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Remarks:
•This application was filed on 14-10-2020 as a divisional application to the application mentioned under INID code 62.
•Claims filed after the date of receipt of the divisional application (Rule 68(4) EPC).

(54) **METHODS OF TREATING CEA-POSITIVE CANCERS USING PD-1 AXIS BINDING ANTAGONISTS AND ANTI-CEA/ANTI-CD3 BISPECIFIC ANTIBODIES**

(57)   The invention provides compositions and methods for treating CEA-positive cancers. The method comprising administering a PD-1 axis binding antagonist and a bispecific antibody that targets CEA and CD3.

EP 3 862 365 A1

Description

FIELD OF THE INVENTION

[0001]   This invention relates to methods of treating CEA-positive cancers by administering a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

BACKGROUND OF THE INVENTION

[0002]   Cancer is one of the leading causes of death worldwide. Despite advances in treatment options, prognosis of patients with advanced cancer remains poor. Consequently, there is a persisting and urgent medical need for optimal therapies to increase survival of cancer patients without causing unacceptable toxicity.

[0003]   Recent results from clinical trials have shown that immune therapies, particularly immune checkpoint inhibitors, can extend the overall survival of cancer patients and lead to durable responses. Despite these promising results, current immune-based therapies are only effective in a proportion of patients and combination strategies are needed to improve therapeutic benefit.

[0004]   Programmed death-ligand 1 (PD-L1) is found on the surface of immune and tumor cells and its expression is induced by interferon gamma (IFNγ). It prevents the immune system from destroying cancer cells by interacting with the inhibitory programmed death-1 (PD-1) and B7.1 receptors on activated T cells, which results in a T-cell inhibitory signal.

[0005]   As a result, therapeutic targeting of PD-1 and other molecules which signal through interactions with PD-1, such as programmed death-ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) are an area of intense interest. PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Okazaki T et al., Intern. Immun. 2007 19(7):813) (Thompson RH et al., Cancer Res 2006, 66(7):3381). Interestingly, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Blood 2009 114(8):1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance (Sharpe et al., Nat Rev 2002) (Keir ME et al., 2008 Annu. Rev. Immunol. 26:677). Therefore, inhibition of the PD-L1/PD-1 interaction may enhance CD8+ T cell-mediated killing of tumors.

[0006]   The inhibition of PD-L1 signaling has been proposed as a means to enhance T cell immunity for the treatment of cancer (e.g., tumor immunity) and infection, including both acute and chronic (e.g., persistent) infection. An optimal therapeutic treatment may combine blockade of PD-1 receptor/ligand interaction with an agent that activates T cells, particularly CD8+ T cells.

[0007]   Dual blockade of PD-1 and PD-L1 has been reported to improve T cell killing of tumor directed by CEA BiTE in an *in vitro* model (Osada et al., Cancer Immunol Immunother (2015) 64(6):677-88).

[0008]   There remains a need for optimal therapies for treating, stabilizing, preventing, and/or delaying development of various cancers in patients.

[0009]   All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE INVENTION

[0010]   In one aspect, provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a human PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

[0011]   In another aspect, provided herein is a method of enhancing immune function in an individual having cancer comprising administering an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

[0012]   In another aspect, provided herein is use of a human PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier.

[0013]   In another aspect, provided herein is use of an anti-CEA/anti-CD3 bispecific antibody in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a human PD-1 axis binding

antagonist and an optional pharmaceutically acceptable carrier.

[0014] In another aspect, provided herein is a composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier.

[0015] In another aspect, provided herein is a composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

[0016] In another aspect, provided herein is a kit comprising a medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0017] In another aspect, provided herein is a kit comprising a first medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a second medicament comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier. In some embodiments, the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament for treating or delaying progression of cancer in an individual.

[0018] In another aspect, provided herein is a kit comprising a medicament comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

[0019] In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is an antibody. In some embodiments, the antibody is a humanized antibody, a chimeric antibody or a human antibody. In some embodiments, the antibody is an antigen binding fragment. In some embodiments, the antigen-binding fragment is selected from the group consisting of Fab, Fab', F(ab')2, and Fv.

[0020] In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2. In some embodiments, the PD-1 binding antagonist is an antibody. In some embodiments, the PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

[0021] In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an anti-PD-L1 antibody. In some embodiments, the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In some embodiments, the anti-PD-LI antibody is MPDL3280A (atezolizumab). In some embodiments, MPDL3280A is administered at a dose of about 800 mg to about 1500 mg every three weeks (e.g., about 1000 mg to about 1300 mg every three weeks, e.g., about 1100 mg to about 1200 mg every three weeks). In some embodiments, MPDL3280A is administered at a dose of about 1200 mg every three weeks. In some embodiments, the anti-PD-LI antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and/or a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24. In some embodiments, the anti-PD-LI antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-LI antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4. In some embodiments, the anti-PD-LI antibody comprises the three heavy chain HVR sequences of antibody YW243.55.S70 and/or the three light chain HVR sequences of antibody YW24355.S70 described in WO 2010/077634 and U.S. Patent No. 8,217,149, which are incorporated herein by reference. In some embodiments, the anti-PD-LI antibody comprises the heavy chain variable region sequence of antibody YW243.55.S70 and/or the light chain variable region sequence of antibody YW24355.S70.

[0022] In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist. In some embodiments, the PD-L2 binding antagonist is an antibody. In some embodiments, the PD-L2 binding antagonist is an immunoadhesin.

[0023] In some embodiments, the PD-1 axis binding antagonist is an antibody (e.g., an anti-PD-1 antibody, an anti-PD-LI antibody, or an anti-PD-L2 antibody) and comprises an aglycosylation site mutation. In some embodiments, the aglycosylation site mutation is a substitution mutation. In some embodiments, the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering). In some embodiments, the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A. In some embodiments, the substitution mutation is a D265A mutation and an N297G mutation. In some embodiments, the aglycosylation site mutation reduces effector function of the antibody. In some embodiments, the PD-1 axis binding antagonist (e.g., an anti-PD-1 antibody, an anti-PD-LI antibody, or an anti-PD-L2 antibody) is a human IgG$_1$ having Asn to Ala substitution at position 297 according to EU numbering.

[0024] In some embodiments of the methods, uses, compositions, and kits described above and herein, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody further comprises a third antigen binding domain that binds to CEA. In some embodiments, the first antigen binding domain binds to a human CD3 polypeptide. In some embodiments, the CD3 polypeptide is a human CD3ε polypeptide or a human CD3γ polypeptide. In some embodiments, the CD3 polypeptide is a human CD3ε polypeptide. In some embodiments, the first antigen binding domain binds to a human CD3ε polypeptide or a human CD3 γ polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits. In some embodiments, the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) and a light chain variable region (V$_L$CD3), and the second (and third, if present) antigen binding domain comprises a heavy chain variable region (V$_H$CEA) and a light chain variable region (V$_L$CEA). In some embodiments, the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) comprising HVR-H1 sequence of SEQ ID NO:44, HVR-H2 sequence of SEQ ID NO:45, and HVR-H3 sequence of SEQ ID NO:46; and/or a light chain variable region (V$_L$CD3) comprising HVR-L1 sequence of SEQ ID NO:47, HVR-L2 sequence of SEQ ID NO:48, and HVR-L3 sequence of SEQ ID NO:49. In some embodiments, the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) comprising the amino acid sequence of SEQ ID NO:50 and/or a light chain variable region (V$_L$CD3) comprising the amino acid sequence of SEQ ID NO:51. In some embodiments, the second (and third, if present) antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V$_L$CEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43. In some embodiment, the second (and third, if present) antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V$_L$CEA) comprising the amino acid sequence of SEQ ID NO:35.

[0025] In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules. In particular such embodiments, the bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the first antigen binding domain is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other) and the second (and third, if present) antigen binding domain is a conventional Fab molecule. In one embodiment, the first antigen binding domain is a crossover Fab molecule wherein the constant domains of the Fab heavy and light chain are exchanged. In one embodiment, the anti-CEA/anti-CD3 bispecific antibody comprises not more than one antigen binding domain that binds to CD3. In one embodiment, the first and the second antigen binding domain are fused to each other, optionally through a peptide linker. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules and (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain.

[0026] In some embodiments, the anti-CEA/anti-CD3 bispecific antibody further comprises an Fc domain composed of a first and a second subunit capable of stable association. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules and (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, and the second antigen

binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain.

[0027] In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules and (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

[0028] In one embodiment the anti-CEA/anti-CD3 bispecific antibody comprises

(i) a first antigen binding domain that binds to CD3, comprising a heavy chain variable region (V_HCD3) comprising HVR-H1 sequence of SEQ ID NO:44, HVR-H2 sequence of SEQ ID NO:45, and HVR-H3 sequence of SEQ ID NO:46; and a light chain variable region (V_LCD3) comprising HVR-L1 sequence of SEQ ID NO:47, HVR-L2 sequence of SEQ ID NO:48, and HVR-L3 sequence of SEQ ID NO:49, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the constant regions, of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding domain that bind to CEA, comprising a heavy chain variable region (V_HCEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and a light chain variable region (V_LCEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit capable of stable association, wherein the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

[0029] In some embodiments, the Fc domain comprised in the anti-CEA/anti-CD3 bispecific antibody is an IgG, specifically an IgG$_1$ or IgG$_4$, Fc domain. In some embodiments, the Fc domain is a human Fc domain. In one embodiment, the Fc domain is a human IgG$_1$ Fc domain.

[0030] In some embodiments, the Fc domain comprised in the anti-CEA/anti-CD3 bispecific antibody comprises a modification promoting the association of the first and the second subunit of the Fc domain. In some such embodiments, in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

[0031] In some embodiments, the Fc domain comprised in the anti-CEA/anti-CD3 bispecific antibody exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG$_1$ Fc domain. In some embodiments, the Fc receptor is an Fcγ receptor and/or the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC). In some such embodiments, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. In one embodiment, said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (EU numbering). In one embodiment, each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G (EU numbering).

[0032] In one embodiment the anti-CEA/anti-CD3 bispecific antibody comprises a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 52, a polypeptide

comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 53, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 54, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 55. In one embodiment, the bispecific antibody comprises a polypeptide comprising the sequence of SEQ ID NO: 52, a polypeptide comprising the sequence of SEQ ID NO: 53, a polypeptide comprising the sequence of SEQ ID NO: 54, and a polypeptide comprising the sequence of SEQ ID NO: 55. (CEA TCB)

[0033] In particular embodiments, the anti-CEA/anti-CD3 bispecific antibody is CEA TCB. In some embodiments, CEA TCB is administered at a dose of about 5 mg to about 400 mg every week (e.g., about 10 mg to about 60 mg every week, e.g., about 10 mg to about 40 mg every week, e.g. about 80 mg to about 200 mg every week, e.g. about 80 mg to about 400 mg every week, or e.g. about 160 mg to 400 mg every week). In some embodiments, CEA TCB is administered at a dose of about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg about 160 mg, particular at least about 80 mg, every week.

[0034] In some embodiments of the methods, uses, compositions and kits described above and herein, the cancer is a CEA-positive cancer. In some embodiments, the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, prostate cancer, or other cancers described herein. In some embodiments, the individual has cancer or has been diagnosed with cancer. In some embodiments, the individual has locally advanced or metastatic cancer or has been diagnosed with locally advanced or metastatic cancer. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the expression of PD-L1 may be determined by an immunohistochemistry (IHC) assay.

[0035] In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment or administration of the human PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody may result in a response in the individual. In some embodiments, the response is a complete response. In some embodiments, the response is a sustained response after cessation of the treatment. In some embodiments, the response is a complete response that is sustained after cessation of the treatment. In other embodiments, the response is a partial response. In some embodiments, the response is a partial response that is sustained after cessation of the treatment.

[0036] In some embodiments of the methods, uses, compositions, and kits described above and herein, the anti-CEA/anti-CD3 bispecific antibody is administered before the PD-1 axis binding antagonist, simultaneous with the PD-1 axis binding antagonist, or after the PD-1 axis binding antagonist. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody is administered after the PD-1 axis binding antagonist, particularly at least one hour after the PD-1 axis binding antagonist when they are both administered on the same day. In some embodiments, the treatment or administration of the human PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody comprises a dosing regimen comprising treatment cycles, wherein the individual is administered, on day 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle, an anti-CEA/anti-CD3 antibody at a dose of about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg about 160 mg, particular at least about 80 mg, each cycle being repeated every 21 days. In some embodiments, the cycle is repeated until there is loss of clinical benefit and/or unacceptable toxicity. In some embodiments, cycles are repeated in which only the PD-1 axis binding antagonist or only the anti-CEA/anti-CD3 antibody is administered.

[0037] In some embodiments, the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody are in the same composition. In some embodiments, the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody are in separate compositions.

[0038] In some embodiments of the methods, uses, compositions, and kits described above and herein, the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody is administered intravenously, intramuscularly, sub-cutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody is administered intravenously. In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment further comprises administering a chemotherapeutic agent for treating or delaying progression of cancer in an individual. In some embodiments, the individual has been treated with a chemotherapeutic agent before the combination treatment with the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody. In some embodiments, the individual treated with the combination of the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody is refractory to a chemotherapeutic agent treatment. In some embodiments, the individual treated with the combination of the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody is intolerant to a chemotherapeutic agent treatment. Some embodiments of the methods, uses, compositions, and kits described throughout the application, further comprise administering a chemotherapeutic agent for treating or delaying progression of cancer.

[0039] In some embodiments of the methods, uses, compositions and kits described above and herein, CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the combination. In some embodiments, the number of CD8 T cells is elevated relative to prior to administration

of the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 antibody. In some embodiments, the CD8 T cell is an antigen-specific CD8 T cell. In some embodiments, Treg function is suppressed relative to prior to the administration of the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 antibody. In some embodiments, T cell exhaustion is decreased relative to prior to the administration of the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 antibody.

[0040] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041]

Figure 1. CEA TCB-mediated lysis of MKN45 cells. Representative graphs of CEA TCB-mediated lysis of MKN-45 tumor cells assessed (A) 24 hours and (B) 48 hours after incubation of tumor cells with human PBMCs (E:T 10:1). The EC50 values of tumor cell killing are: 615 pM (24 h), 362 pM (48 h). Target cell killing was assessed by quantification of LDH released into cell supernatants.

Figure 2. CEA TCB-mediated up-regulation of PD-1 on T cells, and of PD-L1 on surviving tumor cells after tumor cell lysis. Representative graphs of CEA TCB-mediated up-regulation of PD-1 receptor expression on CD8+ T cells (A), CD4+ T cells (B), and of PD-L1 ligand expression on tumor cells that resisted killing (C) analyzed 48 h post incubation of MKN-45 tumor cells with human PBMCs (E:T 10:1). PD-1 and PD-L1 expression was analyzed by flow cytometry.

Figure 3. Tumor growth after treatment with CEA TCB (co-grafting, E:T 5:1). Average tumor burden and standard error of mean (SEM, n = 12); tumor burden was measured by bioluminescence (total flux). CEA TCB was administered IV at doses of 0.5 and 2.5 mg/kg, starting one day (early treatment, panel A) or seven days (late treatment, panel B) after tumor/PBMCs co-grafting and SC injection. Control groups received phosphate-buffer saline (PBS, vehicle), MCSP TCB, or DP47 TCB (as untargeted controls, respectively). In all studies, TCBs were administered twice a week (2q7d). Arrows indicate the day of start of therapy. The curves were compared using non-linear regression analysis. (A) Start of therapy one day after co-grafting with human PBMCs. Curve comparison: vehicle vs. untargeted TCB p-value = 0.65, vehicle vs. CEA TCB (0.5 mg/kg) p-value < 0.0001, vehicle vs. CEA TCB (2.5 mg/kg) p-value < 0.0001, CEA TCB (2.5 mg/kg) vs. CEA TCB (0.5 mg/kg) p-value = 0.91. (B) Start of therapy seven days after co-grafting with human PBMCs. Curve comparison: vehicle vs. untargeted TCB p-value = 0.65, vehicle vs. CEA TCB (0.5 mg/kg) p-value < 0.0001, vehicle vs. CEA TCB (2.5 mg/kg) p-value < 0.0001, CEA TCB (2.5 mg/kg) vs. CEA TCB (0.5 mg/kg) p-value = 0.0008.

Figure 4. Histological analysis of tumors after treatment with CEA TCB (co-grafting, E:T 5:1). Hematoxylin and eosin (H&E, panels A and D) and anti-PD-Ll (panels B, C, E and F) staining of vehicle (panels A-C) and CEA TCB (panels D-F)-treated tumors (2.5 mg/kg, therapy given on Day 7) collected at termination in the co-grafting experiment of LS174T-fluc2 cells with hPBMCs (E:T 5:1) showing strong induction of intra-tumor PD-L1 expression upon CEA TCB-treatment. Panel B and E correspond to the marked area of panel A and D, respectively. Panel C and F correspond to the marked area of panel B and E, respectively.

Figure 5. Tumor weight after treatment with CEA TCB. LS174T-fluc2 cells injected SC and grown until a volume of 100 mm^3 was reached in NOG mice, followed by IP transfer of human PBMCs (10 x 10^6 cells). Three days after PBMCs transfer, mice received bi-weekly IV injections of CEA TCB (2.5 mg/kg), PBS (vehicle), or MCSP TCB (untargeted control, 2.5 mg/kg). Tumor mass was determined at study termination by weighing the explanted tumors. Graphs show average tumor weight and standard error of mean (SEM, 3 ≤ n ≤ 6 mice). ** p < 0.005 using unpaired, two-tailed t-test.

Figure 6. Flow cytometry analysis of tumor-infiltrating leukocytes after treatment with CEA TCB. Flow cytometry analysis of tumor tissues collected at termination in the human colon carcinoma xenograft model with IP transfer of human effector cells. The bar plot shows the number of intra-tumor human CD45+/CD3+ T-cells as assessed by flow cytometry in all treated groups. * p < 0.05 using unpaired, two-tailed t-test.

Figure 7. Immuno PD analysis after treatment with CEA TCB (IP transfer of human PBMCs). Flow cytometry analysis of blood (A-F) and tumor tissues (G-L) collected at Day 18 (corresponding to three days after the third CEA TCB administration) after tumor cell inoculation in the human colon carcinoma xenograft model (IP transfer of human effector cells). Dot plots show the expression of CD69 (B, E, H, K) and PD-1 (C, F, I, L) on CD3 T-cells in both compartments. Representative mice for each group are shown. A-C, G-I: vehicle. D-F, J-L: CEA TCB

Figure 8. CEA TCB-induced tumor growth inhibition in fully humanized mice. Tumor growth inhibition of MKN45 in humanized mice. Tumor volumes at study termination (at day 32 after tumor cell injection) are shown. Each point represents an individual animal. CEA TCB was injected IV (2.5 mg/kg twice weekly), and tumor growth was measured

by caliper. * p < 0.05 (unpaired t-test).

Figure 9. Increase of intra-tumoral human T-cell frequencies and T-cell activation by CEA TCB. Flow cytometric analysis of tumor samples collected at study termination (Day 32). Cells were stained for human antigens (huCD45, huCD3, huPD-1, huCD69, huKi-67, and huGZB). (A, B) Total human T-cell frequencies (A) and the ratio of CD8+ to CD4+ T-cells (B) in the tumor tissue. (C, D) The expression of activation markers in tumor is shown as percent of human CD8+ (C) or CD4+ (D) T-cells. Each point represents an individual animal. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 (unpaired t-test).

Figure 10. Histological analyses of tumor tissues at study termination. Hematoxylin and eosin (H&E, panel A and D) and anti-PD-LI (panel B, C, E and F) staining (Mab SP142) of vehicle (panel A-C) and CEA TCB (panel D-F) -treated tumors exhibiting strong induction of intra-tumoral PD-L1 expression upon CEA TCB treatment. All sections were counterstained with hematoxylin (blue nuclei). Panel B and E correspond to the marked area of panel A and D, respectively. Panel C and F correspond to the marked area of panel B and E, respectively.

Figure 11. In vivo anti-tumor activity upon combination of CEA TCB with antihuman PD-L1 blocking antibody in MKN45 tumor model in fully humanized mice. (A) Average tumor burden and standard error of mean (SEM). Tumor burden was measured by digital caliper 3 times a week. Arrow indicates the day of start of therapy. At day 60: n=7 (vehicle), n=8 (CEA TCB); n=4 (a-PD-LI); n=5 (combination CEA TCB with anti-PD-LI). (B) Time-to-event statistical analysis. The event was defined as reaching 500 mm^3 tumor volume. The pairwise log-rank test was used to compare the following treatment groups, as it can take the different drop-outs into account: vehicle vs. CEA TCB: p= 0.005; CEA TCB vs. anti-PD-LI: p= 0.001; CEA TCB vs. combination of CEA TCB with anti-PD-LI: p= 0.03.

Figure 12. Anti-tumor activity of CEA TCB in the MKN45 model in fully humanized NOG mice. For first line treatment, mice were administered either with vehicle, as control, or with CEA TCB (2.5 mg/kg, administered twice a week for 4 weeks, 7 administrations in total). At study day 34, mice in the CEA TCB-treated group were randomized into two sub-groups: one continued being administered with CEA TCB (2.5 mg/kg twice a week) and the other was administered with CEA TCB (2.5 mg/kg twice a week) in combination with anti-mouse PD-L1 antibody (10 mg/kg, administered once a week) for 5 weeks (11 administrations in total for CEA TCB and 6 administrations in total for anti-PD-LI). ***p<0.0002 two-way ANOVA.

Figure 13. Anti-tumor activity of CEA TCB in the MC38-huCEA model in fully immunocompetent huCEA Tg mice. (A) For first line treatment, mice were administered either with vehicle, as control, with CEA TCB (2.5 mg/kg, administered twice a week for 4 weeks, 7 administrations in total), with anti-mouse PD-L1 antibody (10 mg/kg, administered once a week for 4 weeks, 4 administrations in total), and with a combination of CEA TCB and anti-mouse PD-L1, administered concomitantly with the respective dose and schedules used in the single therapeutic groups. Treatment was administered for 4 weeks. (B) Subcutaneous tumors were surgically removed, and after 5 weeks mice were rechallenged with fresh MC38-huCEA tumors cells injected sub-cutaneously on the opposite flank. As control, groups of naive huCEA Tg mice, either left un-manipulated or submitted to a surgical cut on the flank, were injected sub-cutaneously with MC38-huCEA on the opposite flank.

DETAILED DESCRIPTION

[0042] The inventors of this application demonstrated that a CEA T cell bispecific antibody and anti-PD-LI immune therapy act synergistically in their anti-cancer properties and their combination could provide meaningful clinical benefit in patients with cancer. The data in the application show that the combination of a CEA T cell bispecific antibody (CEA TCB) with anti-PD-LI immune therapy resulted in enhanced inhibition of tumor growth.

[0043] In one aspect, provided herein are methods, compositions and uses for treating or delaying progression of cancer in an individual comprising administering an effective amount of a human PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

[0044] In another aspect, provided herein are methods, compositions and uses for enhancing immune function in an individual having cancer comprising administering an effective amount of a human PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

I. Definitions

[0045] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0046] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0047] As used herein, the terms *"first", "second", "third"* etc. with respect to antigen binding domains etc., are used

for convenience of distinguishing when there is more than one of each type of domain. Use of these terms is not intended to confer a specific order or orientation unless explicitly so stated.

[0048] The term *"about"* as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0049] It is understood that aspects and embodiments of the invention described herein include *"comprising," "consisting,"* and *"consisting essentially of"* aspects and embodiments.

[0050] The term *"PD-1 axis binding antagonist"* refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis - with a result being to restore or enhance T-cell function (e.g., proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. A *"human"* PD-1 axis binding antagonist refers to a PD-1 axis binding antagonist which has the above-described effects on the human PD-1 signaling axis.

[0051] The term *"PD-1 binding antagonist"* refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is MEDI-0680 (AMP-514) described herein. In another specific aspect, a PD-1 binding antagonist is PDR001 described herein. In another specific aspect, a PD-1 binding antagonist is REGN2810 described herein. In another specific aspect, a PD-1 binding antagonist is BGB-108 described herein.

[0052] The term *"PD-L1 binding antagonist"* refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-Ll antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.,* enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-Ll antibody. In a specific aspect, an anti-PD-Ll antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is MPDL3280A (atezolizumab) described herein. In still another specific aspect, an anti-PD-Ll antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-Ll antibody is YW243.55.S70 described herein. In still another specific aspect, an anti-PD-Ll antibody is MEDI4736 (durvalumab) described herein. In still another specific aspect, an anti-PD-Ll antibody is MSB0010718C (avelumab) described herein.

[0053] The term *"PD-L2 binding antagonist"* refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (*e.g.,* enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0054] The term *"dysfunction"* in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both *exhaustion* and/or *anergy* in which antigen

recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

[0055] The term *"dysfunctional"*, as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (*e.g.*, IL-2) and/or target cell killing.

[0056] The term *"anergy"* refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (*e.g.* increase in intracellular Ca^{2+} in the absence of ras-activation). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of costimulation. The unresponsive state can often be overriden by the presence of interleukin-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

[0057] The term *"exhaustion"* refers to T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, etc.).

[0058] *"Enhancing T-cell function"* means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include: increased secretion of γ-interferon from CD8⁺ T-cells, increased proliferation, increased antigen responsiveness (*e.g.,* viral, pathogen, or tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

[0059] A *"T cell dysfunctional disorder"* is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T cell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

[0060] *"Tumor immunity"* refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0061] *"Immunogenicity"* refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

[0062] *"Sustained response"* refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the *beginning* of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

[0063] The term *"pharmaceutical formulation"* refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. *"Pharmaceutically acceptable"* excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0064] As used herein, the term *"treatment"* refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0065] As used herein, *"delaying progression of a disease"* means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late

stage cancer, such as development of metastasis, may be delayed.

[0066] An *"effective amount"* is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.,* slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.,* slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0067] As used herein, "*in conjunction with*" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0068] A *"disorder"* is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

[0069] The terms *"cell proliferative disorder"* and *"proliferative disorder"* refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

[0070] *"Tumor",* as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms *"cancer", "cancerous",* "cell *proliferative* disorder", *"proliferative* disorder" and *"tumor"* are not mutually exclusive as referred to herein.

[0071] The terms *"cancer"* and *"cancerous"* refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.,* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, glioblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers. In some embodiments, the cancer is a CEA-positive cancer.

[0072] The term *"cytotoxic agent"* as used herein refers to any agent that is detrimental to cells (e.g., causes cell death, inhibits proliferation, or otherwise hinders a cellular function). Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.,* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signalling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In one embodiment the cytotoxic agent is a taxane. In one embodiment the taxane is paclitaxel or docetaxel. In one embodiment the cytotoxic agent is a platinum agent. In one embodiment the cytotoxic agent is an antagonist of EGFR. In one embodiment the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (*e.g.,* erlotinib). In one embodiment the cytotoxic agent is a RAF inhibitor. In one embodiment, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In one embodiment the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0073] *"Chemotherapeutic agent"* includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate , salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.,* calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (Angew Chem. Intl. Ed. Engl. 1994 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chlorambucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide

(VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0074] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, iodoxyfene , 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, tripterelin, medroxyprogesterone acetate, diethylstilbestrol, premarin, fluoxymesterone, all transretionic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (e.g., ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN®, rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0075] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG$_1$ λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0076] Chemotherapeutic agent also includes "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (*see,* WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (*see* WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto et al. Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-alpha for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6. 3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns et al., J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.,* EP659439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-

1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimi-dine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluoroph-enyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

[0077] Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrim-idopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phe-nylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphos-tines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules (*e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: US Patent No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[0078] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, am-photericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, fil-grastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methox-salen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, peg-filgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0079] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betam-ethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychlo-roquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor alpha (TNFα) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T cell costimulation blockers such as abatacept (Orencia), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMRA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin alpha (LTa); radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18-OCH3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallo-catechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDRO-CAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as

THERATOPE® vaccine; perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0080]   Chemotherapeutic agents also include non-steroidal anti-inflammatory drugswith analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis, osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0081]   A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0082]   By *"radiation therapy"* is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0083]   A *"subject"* or an *"individual"* for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human. An individual or subject may be a patient.

[0084]   The term *"antibody"* herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.,* bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0085]   An *"isolated"* antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0086]   *"Native antibodies"* are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular

amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0087] The term *"constant domain"* refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[0088] The *"variable region"* or *"variable domain"* of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "V_H." The variable domain of the light chain may be referred to as "V_L." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0089] The term *"variable"* refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0090] The *"light chains"* of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

[0091] The term IgG *"isotype"* or *"subclass"* as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0092] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas et al. Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0093] The terms *"full length antibody," "intact antibody"* and *"whole antibody"* are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[0094] A *"naked antibody"* for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0095] *"Antibody fragments"* comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0096] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0097] *"Fv"* is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0098] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which

have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0099] *"Single-chain Fv"* or *"scFv"* antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.,* Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0100] The term *"diabodies"* refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

[0101] The term *"monoclonal antibody"* as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies comprising the population are identical except for possible mutations, e.g., naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo,* to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0102] The modifier *"monoclonal"* indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (e.g., Kohler and Milstein, Nature, 256:495-97 (1975); Hongo et al., Hybridoma, 14 (3): 253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567), phage-display technologies (see, e.g., Clackson et al., Nature, 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits et al., Nature 362: 255-258 (1993); Bruggemann et al., Year in Immunol. 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., Bio/Technology 10: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild et al., Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

[0103] The monoclonal antibodies herein specifically include *"chimeric"* antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, *e.g.,* U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest.

[0104] *"Humanized"* forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human

residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.,* Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, *e.g.,* Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0105] A *"human antibody"* is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol., 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.,* immunized xenomice (see, *e.g.,* U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0106] A *"species-dependent antibody"* is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "binds specifically" to a human antigen (e.g., has a binding affinity (K_D) value of no more than about 1×10^{-7} M, preferably no more than about 1×10^{-8} M and preferably no more than about 1×10^{-9} M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[0107] The term *"hypervariable region," "HVR,"* or "*HV,*" when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.,* Xu et al., Immunity 13:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.,* Hamers-Casterman et al., Nature 363:446-448 (1993); Sheriff et al., Nature Struct. Biol. 3:733-736 (1996).

[0108] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0109] HVRs may comprise *"extended HVRs"* as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain

residues are numbered according to Kabat et al., *supra,* for each of these definitions.

[0110] *"Framework"* or *"FR"* residues are those variable domain residues other than the HVR residues as herein defined.

[0111] The term *"variable domain residue numbering as in Kabat"* or *"amino acid position numbering as in Kabat,"* and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat et al., *supra.* Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (e.g. residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0112] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The *"EU numbering system"* or *"EU index"* is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat *et al., supra).* The *"EU index as in Kabat"* refers to the residue numbering of the human IgG1 EU antibody.

[0113] The expression *"linear antibodies"* refers to the antibodies described in Zapata et al. (1995 Protein Eng, 8(10):1057-1062). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0114] As use herein, the term *"binds", "specifically binds to"* or is *"specific for"* refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.,* by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of $\leq 1 \mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0115] The term *"bispecific"* means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. Typically, a bispecific antigen binding molecule comprises two antigen binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

[0116] The term *"antigen binding domain"* refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

[0117] A *"Fab molecule"* refers to a protein consisting of the VH and CH1 domain of the heavy chain (the "Fab heavy chain") and the VL and CL domain of the light chain (the "Fab light chain") of an immunoglobulin.

[0118] By a *"crossover"* Fab molecule (also termed "Crossfab") is meant a Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other), i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable domain VL and the heavy chain constant domain 1 CH1 (VL-CH1, in N- to C-terminal direction), and a peptide chain composed of the heavy chain variable domain VH and the light chain constant domain CL (VH-CL, in N- to C-terminal direction). For clarity, in a crossover Fab molecule wherein the variable domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain constant domain 1 CH1 is referred to herein as the "heavy chain" of the (crossover) Fab molecule. Conversely, in a crossover Fab molecule wherein the constant domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain variable domain VH is referred to herein as the "heavy chain" of the (crossover) Fab molecule.

[0119] In contrast thereto, by a *"conventional"* Fab molecule is meant a Fab molecule in its natural format, i.e. comprising a heavy chain composed of the heavy chain variable and constant domains (VH-CH1, in N- to C-terminal direction), and a light chain composed of the light chain variable and constant domains (VL-CL, in N- to C-terminal direction).

[0120] The term *"Fc domain"* or *"Fc region"* herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain.

However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain. Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain (also referred to herein as a "cleaved variant heavy chain"). This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, EU numbering). Therefore, the C-terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (K447), of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (see also above). A *"subunit"* of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

[0121] By *"fused"* is meant that the components (e.g. a Fab molecule and an Fc domain subunit) are linked by peptide bonds, either directly or via one or more peptide linkers.

[0122] A *"modification promoting heterodimerization"* is a manipulation of the peptide backbone or the post-translational modifications of a polypeptide, e.g. an immunoglobulin heavy chain, that reduces or prevents the association of the polypeptide with an identical polypeptide to form a homodimer. A modification promoting heterodimerization as used herein particularly includes separate modifications made to each of two polypeptides desired to form a dimer, wherein the modifications are complementary to each other so as to promote association of the two polypeptides. For example, a modification promoting heterodimerization may alter the structure or charge of one or both of the polypeptides desired to form a dimer so as to make their association sterically or electrostatically favorable, respectively. Heterodimerization occurs between two non-identical polypeptides, e.g. two immunoglobulin heavy chains wherein the variable regions are not the same. In some embodiments, the modification promoting heterodimerization comprises an amino acid mutation, specifically an amino acid substitution. In a particular embodiment, the modification promoting heterodimerization comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two polypeptides desired to form a dimer.

[0123] An *"activating Fc receptor"* is an Fc receptor that following engagement by an Fc region of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Activating Fc receptors include FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32), and FcαRI (CD89).

[0124] The term *"effector functions"* when used in reference to antibodies refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

II. PD-1 Axis Binding Antagonists

[0125] Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. Also provided herein is a method of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. For example, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist. PD-1 (programmed death 1) is also referred to in the art as "programmed cell death 1", PDCD1, CD279 and SLEB2. PD-L1 (programmed death ligand 1) is also referred to in the art as "programmed cell death 1 ligand 1", PDCD1LG1, CD274, B7-H, and PDL1. An exemplary human PD-L1 is shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1. PD-L2 (programmed death ligand 2) is also referred to in the art as "programmed cell death 1 ligand 2", PDCD1LG2, CD273, B7-DC, Btdc, and PDL2. An exemplary human PD-L2 is shown in Uni-ProtKB/Swiss-Prot Accession No. Q9BQ51. In some embodiments, PD-1, PD-L1, and PD-L2 are human PD-1, PD-L1 and PD-L2.

[0126] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect the PD-1 ligand binding partners are PD-L1 and/or PD-L2. In another embodiment, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, PD-L1 binding partners are PD-1 and/or B7-1. In another embodiment, the PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to its binding partners. In a specific aspect, a PD-L2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0127] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (e.g., a human antibody, a hu-

manized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (e.g., an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. In some embodiments, the PD-L1 binding antagonist is anti-PD-Ll antibody. In some embodiments, the anti-PD-Ll antibody is selected from the group consisting of YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). Antibody YW243.55.S70 is an anti-PD-Ll described in WO 2010/077634. MDX-1105, also known as BMS-936559, is an anti-PD-Ll antibody described in WO2007/005874. MEDI4736, is an anti-PD-Ll monoclonal antibody described in WO2011/066389 and US2013/034559. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0128] In some embodiments, the PD-1 axis binding antagonist is an anti-PD-Ll antibody. In some embodiments, the anti-PD-Ll antibody is capable of inhibiting binding between PD-L1 and PD-1 and/or between PD-L1 and B7-1. In some embodiments, the anti-PD-Ll antibody is a monoclonal antibody. In some embodiments, the anti-PD-Ll antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and (Fab')2 fragments. In some embodiments, the anti-PD-Ll antibody is a humanized antibody. In some embodiments, the anti-PD-Ll antibody is a human antibody.

[0129] Examples of anti-PD-Ll antibodies useful for the methods, uses, compositions and kits of this invention, and methods for making thereof are described in PCT patent application WO 2010/077634, WO2007/005874, WO2011/066389, and US2013/034559, which are incorporated herein by reference. The anti-PD-Ll antibodies useful in this invention, including compositions containing such antibodies, may be used in combination with an anti-CEA/anti-CD3 bispecific antibody to treat cancer.

Anti-PDI antibodies

[0130] In some embodiments, the anti-PD-1 antibody is MDX-1106. Alternative names for "MDX-1106" include MDX-1106-04, ONO-4538, BMS-936558 or nivolumab. In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:1 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:2. In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDG

SKRYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVT

VSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA

VLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE

FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT

KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREP

QVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS

FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:1),

and
(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,

at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGI PARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:2).

Anti-PD-LI antibodies

[0131] Anti-PD-LI antibodies described in WO 2010/077634 A1 and US 8,217,149 may be used in the methods, uses, compositions and kits described herein. In some embodiments, the anti-PD-LI antibody comprises a heavy chain variable region sequence of SEQ ID NO:3 and/or a light chain variable region sequence of SEQ ID NO:4. In a still further embodiment, provided is an isolated anti-PD-LI antibody comprising a heavy chain and/or a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG TLVTVSA (SEQ ID NO:3), and

(b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID NO:4).

[0132] In one embodiment, the anti-PD-LI antibody comprises a heavy chain variable region polypeptide comprising an HVR-H1, HVR-H2 and HVR-H3 sequence, wherein:

(a) the HVR-H1 sequence is GFTFSX$_1$SWIH (SEQ ID NO:5);
(b) the HVR-H2 sequence is AWIX2PYGGSX3YYADSVKG (SEQ ID NO:6);
(c) the HVR-H3 sequence is RHWPGGFDY (SEQ ID NO:7);

further wherein: X_1 is D or G; X_2 is S or L; X_3 is T or S. In one specific aspect, X_1 is D; X_2 is S and X_3 is T.
[0133] In another aspect, the polypeptide further comprises variable region heavy chain framework sequences juxta-posed between the HVRs according to the formula: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the framework sequences are VH subgroup III consensus framework. In a still further aspect, at least one of the framework sequences is the following:

HC-FR1 is EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:8)
HC-FR2 is WVRQAPGKGLEWV (SEQ ID NO:9)
HC-FR3 is RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)
HC-FR4 is WGQGTLVTVSA (SEQ ID NO:11).

[0134] In a still further aspect, the heavy chain polypeptide is further combined with a variable region light chain comprising an HVR-L1, HVR-L2 and HVR-L3, wherein:

(a) the HVR-L1 sequence is RASQX$_4$X$_5$X$_6$TX$_7$X$_8$A (SEQ ID NO:12);
(b) the HVR-L2 sequence is SASX$_9$LX$_{10}$S, (SEQ ID NO:13);
(c) the HVR-L3 sequence is QQX$_{11}$X$_{12}$X$_{13}$X$_{14}$PX$_{15}$T (SEQ ID NO:14);

wherein: X$_4$ is D or V; X$_5$ is V or I; X$_6$ is S or N; X$_7$ is A or F; X$_8$ is V or L; X$_9$ is F or T; X$_{10}$ is Y or A; X$_{11}$ is Y, G, F, or S; X$_{12}$ is L, Y, F or W; X$_{13}$ is Y, N, A, T, G, F or I; X$_{14}$ is H, V, P, T or I; X$_{15}$ is A, W, R, P or T. In a still further aspect, X$_4$ is D; X$_5$ is V; X$_6$ is S; X$_7$ is A; X$_8$ is V; X$_9$ is F; X$_{10}$ is Y; X$_{11}$ is Y; X$_{12}$ is L; X$_{13}$ is Y; X$_{14}$ is H; X$_{15}$ is A.

[0135] In a still further aspect, the light chain further comprises variable region light chain framework sequences juxtaposed between the HVRs according to the formula: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the framework sequences are VL kappa I consensus framework. In a still further aspect, at least one of the framework sequence is the following:

LC-FR1 is DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)
LC-FR2 is WYQQKPGKAPKLLIY (SEQ ID NO:16)
LC-FR3 is GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)
LC-FR4 is FGQGTKVEIKR (SEQ ID NO:18).

[0136] In another embodiment, provided is an isolated anti-PD-LI antibody or antigen binding fragment comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain comprises and HVR-H1, HVR-H2 and HVR-H3, wherein further:

(i) the HVR-H1 sequence is GFTFSX$_1$SWIH; (SEQ ID NO:5)
(ii) the HVR-H2 sequence is AWIX2PYGGSX3YYADSVKG (SEQ ID NO:6)
(iii) the HVR-H3 sequence is RHWPGGFDY, and (SEQ ID NO:7)

(b) the light chain comprises and HVR-L1, HVR-L2 and HVR-L3, wherein further:

(i) the HVR-L1 sequence is RASQX$_4$X$_5$X$_6$TX$_7$X$_8$A (SEQ ID NO: 12)
(ii) the HVR-L2 sequence is SASX$_9$LX$_{10}$S; and (SEQ ID NO:13)
(iii) the HVR-L3 sequence is QQX$_{11}$X$_{12}$X$_{13}$X$_{14}$PX$_{15}$T; (SEQ ID NO:14)

wherein: X$_1$ is D or G; X$_2$ is S or L; X$_3$ is T or S; X$_4$ is D or V; X$_5$ is V or I; X$_6$ is S or N; X$_7$ is A or F; X$_8$ is V or L; X$_9$ is F or T; X$_{10}$ is Y or A; X$_{11}$ is Y, G, F, or S; X$_{12}$ is L, Y, F or W; X$_{13}$ is Y, N, A, T, G, F or I; X$_{14}$ is H, V, P, T or I; X$_{15}$ is A, W, R, P or T. In a specific aspect, X$_1$ is D; X$_2$ is S and X$_3$ is T. In another aspect, X$_4$ is D; X$_5$ is V; X$_6$ is S; X$_7$ is A; X$_8$ is V; X$_9$ is F; X$_{10}$ is Y; X$_{11}$ is Y; X$_{12}$ is L; X$_{13}$ is Y; X$_{14}$ is H; X$_{15}$ is A. In yet another aspect, X$_1$ is D; X$_2$ is S and X$_3$ is T, X$_4$ is D; X$_5$ is V; X$_6$ is S; X$_7$ is A; X$_8$ is V; X$_9$ is F; X$_{10}$ is Y; X$_{11}$ is Y; X$_{12}$ is L; X$_{13}$ is Y; X$_{14}$ is H and X$_{15}$ is A.

[0137] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0138] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific

aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0139]　In yet another embodiment, provided is an anti-PD-Ll antibody comprising a heavy chain and a light chain variable region sequence, wherein:

> (a) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, or
> (b) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0140]　In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and 11. In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0141]　In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0142]　In another further embodiment, provided is an isolated anti-PD-Ll antibody comprising a heavy chain and a light chain variable region sequence, wherein:

> (a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG

STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG

TLVTVSS (SEQ ID NO:25), and/or

> (b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG

VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID

NO:4).

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-

L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSS (SEQ ID NO:27).

In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18.

[0143] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect, the minimal effector function results from production in prokaryotic cells. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0144] In a further aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In a still further aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences is the following:

    HC-FR1 EVQLVESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO:29)
    HC-FR2 WVRQAPGKGLEWVA (SEQ ID NO:30)
    HC-FR3 RFTISADTSKNTAYLQMNSLRAEDTAVYYCAR (SEQ ID NO:10)
    HC-FR4 WGQGTLVTVSS (SEQ ID NO:27).

[0145] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences is the following:

    LC-FR1 DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO:15)
    LC-FR2 WYQQKPGKAPKLLIY (SEQ ID NO:16)
    LC-FR3 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:17)
    LC-FR4 FGQGTKVEIK (SEQ ID NO:28).

[0146] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0147] In yet another embodiment, provided is an anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein:

    (c) the heavy chain further comprises an HVR-H1, HVR-H2 and an HVR-H3 sequence having at least 85% sequence identity to GFTFSDSWIH (SEQ ID NO:19), AWISPYGGSTYYADSVKG (SEQ ID NO:20) and RHWPGGFDY (SEQ ID NO:21), respectively, and/or
    (d) the light chain further comprises an HVR-L1, HVR-L2 and an HVR-L3 sequence having at least 85% sequence identity to RASQDVSTAVA (SEQ ID NO:22), SASFLYS (SEQ ID NO:23) and QQYLYHPAT (SEQ ID NO:24), respectively.

In a specific aspect, the sequence identity is 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%.

[0148] In another aspect, the heavy chain variable region comprises one or more framework sequences juxtaposed between the HVRs as: (HC-FR1)-(HVR-H1)-(HC-FR2)-(HVR-H2)-(HC-FR3)-(HVR-H3)-(HC-FR4), and the light chain variable regions comprises one or more framework sequences juxtaposed between the HVRs as: (LC-FR1)-(HVR-L1)-(LC-FR2)-(HVR-L2)-(LC-FR3)-(HVR-L3)-(LC-FR4). In yet another aspect, the framework sequences are derived from human consensus framework sequences. In a still further aspect, the heavy chain framework sequences are derived from a Kabat subgroup I, II, or III sequence. In a still further aspect, the heavy chain framework sequence is a VH subgroup III consensus framework. In a still further aspect, one or more of the heavy chain framework sequences are set forth as SEQ ID NOs:8, 9, 10 and WGQGTLVTVSSASTK (SEQ ID NO:31).

[0149] In a still further aspect, the light chain framework sequences are derived from a Kabat kappa I, II, II or IV subgroup sequence. In a still further aspect, the light chain framework sequences are VL kappa I consensus framework. In a still further aspect, one or more of the light chain framework sequences are set forth as SEQ ID NOs:15, 16, 17 and 18. In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, IgG3. In a still further aspect, the murine constant region is IgG2A. In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an "effector-less Fc mutation" or aglycosylation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region.

[0150] In a still further embodiment, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG

STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG

TLVTVSS (SEQ ID NO:25),

or
(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG

VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID

NO:4).

[0151] In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:25. In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:25. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0152] In a still further embodiment, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light

chain variable region sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTK (SEQ ID NO:26),

or
(b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKR (SEQ ID
NO:4).

[0153] In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:4. In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain variable region sequence, wherein the light chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:4 and the heavy chain variable region sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:26. In some embodiments, one, two, three, four or five amino acid residues at the N-terminal of the heavy and/or light chain may be deleted, substituted or modified.

[0154] In a still further embodiment, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain sequence, wherein:

(a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID
NO:32), and/or

   (b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33)

[0155] In a still further embodiment, provided is an isolated anti-PD-LI antibody comprising a heavy chain and a light chain sequence, wherein:

   (a) the heavy chain sequence has at least 85% sequence identity to the heavy chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ
ID NO:56), and/or

   (b) the light chain sequences has at least 85% sequence identity to the light chain sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33).

[0156] In some embodiments, provided is an isolated anti-PD-Ll antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33. In some embodiments, provided is an isolated anti-PD-Ll antibody comprising a heavy chain and a light chain sequence, wherein the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 56. In some embodiments, provided is an isolated anti-PD-Ll antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32 or 56. In some embodiments, provided is an isolated anti-PD-L1 antibody comprising a heavy chain and a light chain sequence, wherein the light chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:33 and the heavy chain sequence has at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:32.

[0157] In some embodiments, the isolated anti-PD-Ll antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site another amino acid residue (e.g., glycine, alanine or a conservative substitution).

[0158] In any of the embodiments herein, the isolated anti-PD-Ll antibody can bind to a human PD-L1, for example a human PD-L1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof.

[0159] In a still further embodiment, provided is an isolated nucleic acid encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-PDLl antibodies. In a still further specific aspect, the vector is in a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese hamster ovary (CHO) cell.

[0160] The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-PDLl antibodies or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

III. Anti-CEA/anti-CD3 bispecific Antibodies

[0161] Provided herein is a method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. Also provided herein is a method of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

[0162] Provided herein are bispecific antibodies that bind to a human carcinoembryonic antigen (CEA). Alternative names for "CEA" include CEACAM5. The term "CEA" as used herein, refers to any native CEA from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length" and unprocessed CEA as well as any form of CEA that results from processing in the cell (e.g., mature protein). The term also encompasses naturally occurring variants and isoforms of CEA, e.g., splice variants or allelic variants. In one embodiment, CEA is human CEA. The amino acid sequence of human CEA is shown in UniProt (www.uniprot.org) accession no. P06731,

or NCBI (www.ncbi.nlm.nih.gov/) RefSeq NP_004354.2.

[0163] In some embodiments, the antigen binding domain of a bispecific antibody that binds to CEA comprises a heavy chain variable region (V$_H$CEA) comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTEFGMNWVRQAPGQGLEWMGWINTK

TGEATYVEEFKGRVTFTTDTSTSTAYMELRSLRSDDTAVYYCARWDFAYYVEAMD

YWGQGTTVTVSS (SEQ ID NO:34),

and /or a light chain variable region (V$_L$CEA) comprising the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCKASAAVGTYVAWYQQKPGKAPKLLIYSASYRKRG

VPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYYTYPLFTFGQGTKLEIK (SEQ ID

NO:35).

[0164] In some embodiments, the antigen binding domain of a bispecific antibody that binds to CEA comprises a heavy chain variable region comprising an HVR-H1 sequence of EFGMN (SEQ ID NO:38), an HVR-H2 sequence of WINTKT-GEATYVEEFKG (SEQ ID NO:39), and an HVR-H3 sequence of WDFAYYVEAMDY (SEQ ID NO:40) and a light chain variable region comprising an HVR-L1 sequence of KASAAVGTYVA (SEQ ID NO:41), an HVR-L2 sequence of SASYRKR (SEQ ID NO:42), and an HVR-L3 sequence of HQYYTYPLFT (SEQ ID NO:43).

[0165] In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds a human CD3 polypeptide, and a second antigen binding domain that binds CEA. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody further comprises a third antigen binding domain that binds CEA. In some embodiments, the second and the third antigen binding domain are identical (i.e. have the same amino acid sequences)

[0166] CD3 (cluster of differentiation 3) T-cell co-receptor is a protein complex and is composed of four distinct chains. In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with the T-cell receptor (TCR) and the ζ-chain to generate an activation signal in T lymphocytes. The TCR, ζ-chain, and CD3 molecules together form the TCR complex. The term "CD3" as used herein, refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g. humans), non-human primates (e.g. cynomolgus monkeys) and rodents (e.g. mice and rats), unless otherwise indicated. The term encompasses "full-length" and unprocessed protein as well as any form of the protein or one or more of the CD3 chains (polypeptides) that result from processing in the cell (e.g., mature polypeptides). The term also encompasses naturally occurring variants and isoforms of CD3, e.g., splice variants or allelic variants. For example, descriptions of CD3y, CD3δ, and CD3ε chains and sequences are provided at www.uniprot.org/uniprot/P04234, www.uniprot.org/uniprot/P07766, and www.uniprot.org/uniprot/P09693. In one embodiment, CD3 is human CD3, particularly the epsilon subunit of human CD3 (CD3ε). The amino acid sequence of human CD3ε is shown in UniProt (www.uniprot.org) accession no. P07766 (version 144), or NCBI (www.nc-bi.nlm.nih.gov/) RefSeq NP_000724.1. The amino acid sequence of cynomolgus [Macaca fascicularis] CD3ε is shown in NCBI GenBank no. BAB71849.1.

[0167] In some embodiments, the bispecific antibody binds to a human CD3 epsilon (CD3ε) polypeptide. In some embodiments, the bispecific antibody binds to a human CD3 epsilon polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits. In some embodiments, the bispecific antibody binds to a human CD3 gamma (CD3γ) polypeptide. In some embodiments, the bispecific antibody binds a human CD3 gamma polypeptide in native T-cell receptor (TCR) complex in association with other TCR subunits.

[0168] In one aspect, assays are provided for identifying anti-CD3 antibodies thereof having biological activity. Biological activity may include, for example, binding to a CD3 polypeptide (e.g., CD3 on the surface of a T cell), or a peptide fragment thereof, either *in vivo, in vitro,* or *ex vivo.* In the case of a multispecific *(e.g.,* bispecific) anti-CD3 antibody of the invention (e.g., a TCB antibody having an anti-CEA binding domain and a binding domain that recognizes a CD3 polypeptide), biological activity may also include, for example, effector cell activation (e.g., T cell (e.g., CD8+ and/or CD4+ T cell) activation), effector cell population expansion (i.e., an increase in T cell count), target cell population reduction (i.e., a decrease in the population of cells expressing CEA on their cell surfaces), and/or target cell killing. Antibodies having such biological activity *in vivo* and/or *in vitro* are provided. In certain embodiments, an antibody of the invention is tested for such biological activity.

[0169] In some embodiments, the antigen binding domain(s) of a bispecific antibody that binds to a CEA comprises

a heavy chain variable region (V$_H$CEA) comprising the amino acid sequence of SEQ ID NO:34, and a light chain variable region (V$_L$CEA) comprising the amino acid sequence of SEQ ID NO:35. In some embodiments, the antigen binding domain(s) that binds to a CEA comprises a heavy chain variable region comprising an HVR-H1 sequence of EFGMN (SEQ ID NO:38), an HVR-H2 sequence of WINTKTGEATYVEEFKG (SEQ ID NO:39), and an HVR-H3 sequence of WDFAYYVEAMDY (SEQ ID NO:40) and comprises a light chain variable region comprising an HVR-L1 sequence of KASAAVGTYVA (SEQ ID NO:41), an HVR-L2 sequence of SASYRKR (SEQ ID NO:42), and an HVR-L3 sequence of HQYYTYPLFT (SEQ ID NO:43).

[0170]   In some embodiments, the antigen binding domain of a bispecific antibody that binds to a CD3 comprises a heavy chain variable region (V$_H$CD3) comprising the amino acid sequence of SEQ ID NO:50, and a light chain variable region (V$_L$CD3) comprising the amino acid sequence of SEQ ID NO:51. In some embodiments, the antigen binding domain that binds to a CD3 comprises a heavy chain variable region comprising an HVR-H1 sequence of TYAMN (SEQ ID NO:44), an HVR-H2 sequence of RIRSKYNNYATYYADSVKG (SEQ ID NO:45), and an HVR-H3 sequence of HGN-FGNSYVSWFAY (SEQ ID NO:46) and comprises a light chain comprising an HVR-L1 sequence of GSSTGAVTTSNYAN (SEQ ID NO:47), an HVR-L2 sequence of GTNKRAP (SEQ ID NO:48), and an HVR-L3 sequence of ALWYSNLWV (SEQ ID NO:49).

[0171]   In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules (e.g. conventional or crossover Fab molecules). In particular such embodiments, the bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the first antigen binding domain is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other) and the second (and third, if present) antigen binding domain is a conventional Fab molecule. In one embodiment, the first antigen binding domain is a crossover Fab molecule wherein the constant domains of the Fab heavy and light chain are exchanged. In one embodiment, the anti-CEA/anti-CD3 bispecific antibody comprises not more than one antigen binding domain that binds to CD3, i.e. provides monovalent binding to CD3. In one embodiment, the first and the second antigen binding domain are fused to each other, optionally through a peptide linker. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3 and a second and optionally a third antigen binding domain that binds to CEA, wherein the antigen binding domains are Fab molecules and (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain. Optionally, the Fab light chain of the first antigen binding domain and the Fab light chain of the second antigen binding domain may additionally be fused to each other.

[0172]   In some embodiments, the anti-CEA/anti-CD3 bispecific antibody further comprises an Fc domain composed of a first and a second subunit capable of stable association. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, a second antigen binding domain that binds to CEA, and an Fc domain composed of a first and a second subunit capable of stable association, wherein the antigen binding domains are Fab molecules and wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody essentially consists of a first antigen binding domain that binds to CD3, a second antigen binding domain that binds to CEA, an Fc domain composed of a first and a second subunit capable of stable association, and optionally one or more peptide linkers, wherein the antigen binding domains are Fab molecules and wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain.

[0173]   In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, a second and a third antigen binding domain that binds to CEA, and an Fc domain composed of a first

and a second subunit capable of stable association, wherein the antigen binding domains are Fab molecules and wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. In a particular embodiment, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody essentially consists of a first antigen binding domain that binds to CD3, a second and a third antigen binding domain that binds to CEA, an Fc domain composed of a first and a second subunit capable of stable association, and optionally one or more peptide linkers, wherein the antigen binding domains are Fab molecules and wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

[0174]   In one embodiment the anti-CEA/anti-CD3 bispecific antibody comprises (

i) a first antigen binding domain that binds to CD3, comprising a heavy chain variable region (V_HCD3) comprising HVR-H1 sequence of SEQ ID NO:44, HVR-H2 sequence of SEQ ID NO:45, and HVR-H3 sequence of SEQ ID NO:46; and a light chain variable region (V_LCD3) comprising HVR-L1 sequence of SEQ ID NO:47, HVR-L2 sequence of SEQ ID NO:48, and HVR-L3 sequence of SEQ ID NO:49, wherein the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the constant regions, of the Fab light chain and the Fab heavy chain are exchanged;
(ii) a second and a third antigen binding domain that bind to CEA, comprising a heavy chain variable region (V_HCEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and a light chain variable region (V_LCEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43, wherein the second and third antigen binding moiety are each a Fab molecule, particularly a conventional Fab molecule;
(iii) an Fc domain composed of a first and a second subunit capable of stable association, wherein the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

[0175]   The Fab molecules may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, nonimmunogenic peptide linkers include, for example, $(G_4S)_n$, $(SG_4)_n$, $(G_4S)_n$ or $G_4(SG_4)_n$ peptide linkers. "n" is generally an integer from 1 to 10, typically from 2 to 4. In one embodiment said peptide linker has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is $(GxS)_n$ or $(GxS)_nG_m$ with G=glycine, S=serine, and (x=3, n= 3, 4, 5 or 6, and m=0, 1, 2 or 3) or (x=4, n=2, 3, 4 or 5 and m= 0, 1, 2 or 3), in one embodiment x=4 and n=2 or 3, in a further embodiment x=4 and n=2. In one embodiment said peptide linker is $(G_4S)_2$. A particularly suitable peptide linker for fusing the Fab light chains of the first and the second antigen binding domain to each other is (G4S)2. An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second antigen binding domain comprises the sequence (D)-$(G_4S)_2$. Another suitable such linker comprises the sequence $(G_4S)_4$. Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.
[0176]   In some embodiments, the anti-CEA/anti-CD3 bispecific antibody comprises (i) a polypeptide wherein the Fab heavy chain of a first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of

a second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with a first Fc domain subunit ($VH_{(1)}$-$CH1_{(1)}$-$VH_{(2)}$-$CL_{(2)}$-CH2-CH3(-CH4)), (ii) a crossover Fab light chain polypeptide of the second Fab molecule, wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule ($VL_{(2)}$-$CH1_{(2)}$), (iii) the Fab light chain polypeptide of the first Fab molecule ($VL_{(1)}$-$CL_{(1)}$), (iv) a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with a second Fc domain subunit ($VH_{(3)}$-$CH1_{(3)}$-CH2-CH3(-CH4)) and (v) the Fab light chain polypeptide of the third Fab molecule ($VL_{(3)}$-$CL_{(3)}$). In certain embodiments, the Fab light chain polypeptide of the first and the third Fab molecule are identical. In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond. In certain embodiments, the first and the third Fab molecule bind to CEA, and the second Fab molecule binds to CD3.

[0177] In one embodiment the anti-CEA/anti-CD3 bispecific antibody comprises a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 52, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 53, a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 54, and a polypeptide comprising a sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 55. In one embodiment, the bispecific antibody comprises a polypeptide comprising the sequence of SEQ ID NO: 52, a polypeptide comprising the sequence of SEQ ID NO: 53, a polypeptide comprising the sequence of SEQ ID NO: 54, and a polypeptide comprising the sequence of SEQ ID NO: 55.

Fc domain

[0178] The anti-CEA/anti-CD3 bispecific antibody used in the present invention may comprise an Fc domain which consists of a pair of polypeptide chains comprising heavy chain domains of an antibody molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other.

[0179] In one embodiment, the Fc domain is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG_1 Fc domain. In another embodiment the Fc domain is an IgG_4 Fc domain. In a more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising an amino acid substitution at position S228 (EU numbering), particularly the amino acid substitution S228P. This amino acid substitution reduces *in vivo* Fab arm exchange of IgG4 antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In a further particular embodiment the Fc domain is human. An exemplary sequence of a human IgG_1 Fc region is given in SEQ ID NO: 57.

Fc domain modifications promoting heterodimerization

[0180] The anti-CEA/anti-CD3 bispecific antibody used in the present invention may comprise different components (e.g. antigen binding domains) fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain are typically comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of such antibodies in recombinant production, it will thus be advantageous to introduce in the Fc domain of the antibody a modification promoting the association of the desired polypeptides.

[0181] Accordingly, in particular embodiments the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

[0182] There exist several approaches for modifications in the CH3 domain of the Fc domain in order to enforce heterodimerization, which are well described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012058768, WO 2013157954, WO 2013096291. Typically, in all such approaches the CH3 domain of the first subunit of the Fc domain and the CH3 domain of the second subunit of the Fc domain are both engineered in a complementary manner so that each CH3 domain (or the heavy chain comprising it) can no longer homodimerize with itself but is forced to heterodimerize with the complementarily engineered other CH3 domain (so that the first and second CH3 domain heterodimerize and no homodimers between the two first or the two second CH3 domains are formed).

[0183] In a specific embodiment said modification promoting the association of the first and the second subunit of the Fc domain is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain.

[0184] The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

[0185] Accordingly, in a particular embodiment, in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

[0186] Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W).

[0187] Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V).

[0188] The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, e.g. by site-specific mutagenesis, or by peptide synthesis.

[0189] In a specific embodiment, in the CH3 domain of the first subunit of the Fc domain (the "knobs" subunit) the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain (the "hole" subunit) the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In one embodiment, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (EU numbering).

[0190] In yet a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (EU numbering). Introduction of these two cysteine residues results in formation of a disulfide bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

[0191] In a particular embodiment, the first subunit of the Fc domain comprises amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises amino acid substitutions Y349C, T366S, L368A and Y407V (EU numbering).

[0192] In a particular embodiment the antigen binding domain that binds to CD3 in the anti-CEA/anti-CD3 bispecific antibody described herein is fused to the first subunit of the Fc domain (comprising the "knob" modification). Without wishing to be bound by theory, fusion of the CD3 binding domain to the knob-containing subunit of the Fc domain will (further) minimize the generation of bispecific antibodies comprising two CD3 binding domains (steric clash of two knob-containing polypeptides).

[0193] Other techniques of CH3-modification for enforcing the heterodimerization are contemplated as alternatives according to the invention and are described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954, WO 2013/096291.

[0194] In one embodiment the heterodimerization approach described in EP 1870459 A1, is used alternatively. This approach is based on the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3 domain interface between the two subunits of the Fc domain. One preferred embodiment are amino acid mutations R409D; K370E in one of the two CH3 domains (of the Fc domain) and amino acid mutations D399K; E357K in the other one of the CH3 domains of the Fc domain (EU numbering).

[0195] In another embodiment the antibody comprises amino acid mutation T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (EU numbering).

[0196] In another embodiment the antibody comprises amino acid mutations S354C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations Y349C, T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, or the antibody comprises amino acid mutations Y349C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations S354C, T366S, L368A, Y407V in the CH3 domains of

the second subunit of the Fc domain and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (all numberings according to EU numbering).

[0197] In one embodiment the heterodimerization approach described in WO 2013/157953 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutation T366K and a second CH3 domain comprises amino acid mutation L351D (EU numbering). In a further embodiment the first CH3 domain comprises further amino acid mutation L351K. In a further embodiment the second CH3 domain comprises further an amino acid mutation selected from Y349E, Y349D and L368E (preferably L368E) (EU numbering).

[0198] In one embodiment the heterodimerization approach described in WO 2012/058768 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment the second CH3 domain comprises a further amino acid mutation at position T411, D399, S400, F405, N390, or K392, e.g. selected from a) T411N, T411R, T411Q, T411K, T411D, T411E or T411W, b) D399R, D399W, D399Y or D399K, c) S400E, S400D, S400R, or S400K, d) F405I, F405M, F405T, F405S, F405V or F405W, e) N390R, N390K or N390D, f) K392V, K392M, K392R, K392L, K392F or K392E (EU numbering). In a further embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366V, K409F. In a further embodiment a first CH3 domain comprises amino acid mutation Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment the second CH3 domain further comprises amino acid mutations K392E, T411E, D399R and S400R (EU numbering).

[0199] In one embodiment the heterodimerization approach described in WO 2011/143545 is used alternatively, e.g. with the amino acid modification at a position selected from the group consisting of 368 and 409 (EU numbering).

[0200] In one embodiment the heterodimerization approach described in WO 2011/090762, which also uses the knobs-into-holes technology described above, is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutation T366W and a second CH3 domain comprises amino acid mutation Y407A. In one embodiment a first CH3 domain comprises amino acid mutation T366Y and a second CH3 domain comprises amino acid mutation Y407T (EU numbering).

[0201] In one embodiment the antibody or its Fc domain is of IgG2 subclass and the heterodimerization approach described in WO 2010/129304 is used alternatively.

[0202] In an alternative embodiment a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable. In one such embodiment a first CH3 domain comprises amino acid substitution of K392 or N392 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K392D or N392D) and a second CH3 domain comprises amino acid substitution of D399, E356, D356, or E357 with a positively charged amino acid (e.g. lysine (K) or arginine (R), preferably D399K, E356K, D356K, or E357K, and more preferably D399K and E356K). In a further embodiment the first CH3 domain further comprises amino acid substitution of K409 or R409 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K409D or R409D). In a further embodiment the first CH3 domain further or alternatively comprises amino acid substitution of K439 and/or K370 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D)) (all numberings according to EU numbering).

[0203] In yet a further embodiment the heterodimerization approach described in WO 2007/147901 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutations K253E, D282K, and K322D and a second CH3 domain comprises amino acid mutations D239K, E240K, and K292D (EU numbering).

[0204] In still another embodiment the heterodimerization approach described in WO 2007/110205 can be used alternatively.

[0205] In one embodiment, the first subunit of the Fc domain comprises amino acid substitutions K392D and K409D, and the second subunit of the Fc domain comprises amino acid substitutions D356K and D399K (EU numbering).

Fc domain modifications reducing Fc receptor binding and/or effector function

[0206] The Fc domain confers to an antibody, such as a bispecific antibody, favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the antibody to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with other immunostimulatory properties the antibody may have and the long half-life of the antibody, results in excessive activation of cytokine receptors and severe side effects upon systemic administration.

[0207] Accordingly, in particular embodiments, the Fc domain of the anti-CEA/anti-CD3 bispecific antibody exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG$_1$ Fc domain. In one such embodiment the Fc domain (or the molecule, e.g. antibody, comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG$_1$ Fc domain (or a corresponding molecule comprising a native IgG$_1$ Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG$_1$ Fc domain domain (or a corresponding molecule comprising a native IgG$_1$ Fc domain). In one embodiment, the Fc domain (or the molecule, e.g. antibody, comprising said Fc domain) does not substantially bind to an Fc receptor and/or induce effector function. In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one embodiment the effector function is one or more selected from the group of CDC, ADCC, ADCP, and cytokine secretion. In a particular embodiment the effector function is ADCC. In one embodiment the Fc domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG$_1$ Fc domain domain. Substantially similar binding to FcRn is achieved when the Fc domain (or the molecule, e.g. antibody, comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG$_1$ Fc domain (or the corresponding molecule comprising a native IgG$_1$ Fc domain) to FcRn.

[0208] In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In particular embodiments, the Fc domain comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to an Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the molecule, e.g. antibody, comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to a corresponding molecule comprising a non-engineered Fc domain. In a particular embodiment the Fc receptor is an Fcγ receptor. In some embodiments the Fc receptor is a human Fc receptor. In some embodiments the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. Preferably, binding to each of these receptors is reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the molecule, e.g. antibody, comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or a corresponding molecule comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or molecule (e.g. antibody) comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced crosslinking of target-bound antibodies, reduced dendritic cell maturation, or reduced T cell priming. In one embodiment the reduced effector function is one or more selected from the group of reduced CDC, reduced ADCC, reduced ADCP, and reduced cytokine secretion. In a particular embodiment the reduced effector function is reduced ADCC. In one embodiment the reduced ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or a corresponding molecule comprising a non-engineered Fc domain).

[0209] In one embodiment the amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (EU numbering). In a more specific embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (EU numbering). In some embodiments the Fc domain comprises the amino acid substitutions L234A and L235A (EU numbering). In one such embodiment, the Fc domain is an IgG$_1$ Fc domain, particularly a human IgG$_1$ Fc domain. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is P329A or P329G, particularly P329G (EU numbering). In one embodiment

the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (EU numbering). In a more specific embodiment the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 EU numbering). In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA"). In one such embodiment, the Fc domain is an IgG$_1$ Fc domain, particularly a human IgG$_1$ Fc domain. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor (as well as complement) binding of a human IgG$_1$ Fc domain, as described in PCT publication no. WO 2012/130831, incorporated herein by reference in its entirety. WO 2012/130831 also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

[0210]   IgG4 antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG$_1$ antibodies. Hence, in some embodiments the Fc domain is an IgG4 Fc domain, particularly a human IgG4 Fc domain. In one embodiment the IgG4 Fc domain comprises amino acid substitutions at position S228, specifically the amino acid substitution S228P (EU numbering). To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG$_4$ Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E (EU numbering). In another embodiment, the IgG$_4$ Fc domain comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G (EU numbering). In a particular embodiment, the IgG$_4$ Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G (EU numbering). Such IgG4 Fc domain mutants and their Fcγ receptor binding properties are described in PCT publication no. WO 2012/130831, incorporated herein by reference in its entirety.

[0211]   In a particular embodiment the Fc domain exhibiting reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG$_1$ Fc domain, is a human IgG$_1$ Fc domain comprising the amino acid substitutions L234A, L235A and optionally P329G, or a human IgG4 Fc domain comprising the amino acid substitutions S228P, L235E and optionally P329G (EU numbering).

[0212]   In certain embodiments N-glycosylation of the Fc domain has been eliminated. In one such embodiment the Fc domain comprises an amino acid mutation at position N297, particularly an amino acid substitution replacing asparagine by alanine (N297A) or aspartic acid (N297D) or glycine (N297G) (EU numbering).

[0213]   In addition to the Fc domains described hereinabove and in PCT publication no. WO 2012/130831, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056) (EU numbering). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0214]   Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

[0215]   Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. Alternatively, binding affinity of Fc domains or molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor.

[0216]   Effector function of an Fc domain, or a molecule (e.g. an antibody) comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo,* e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

[0217]   In some embodiments, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have reduced effector function, said reduced effector function includes reduced CDC. C1q binding assays may be carried out to determine whether the Fc domain, or molecule (e.g. antibody) comprising the Fc domain, is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

[0218] In some embodiments, the bispecific antibody is a single-chain bispecific antibody comprising the first antigen binding domain and the second antigen binding domain. In some embodiments, the single-chain bispecific antibody comprises variable regions, as arranged from N-terminus to C-terminus, selected from the group consisting of (1) V_HCEA-V_LCEA-V_HCD3-V_LCD3, (2) V_HCD3-V_LCD3-V_HCEA-V_LCEA, (3) V_HCD3-V_LCD3-V_LCEA-V_HCEA, (4) V_HCEA-V_LCEA-V_LCD3-V_HCD3, (5) V_LCEA-V_HCEA-V_HCD3-V_LCD3, and (6) V_LCD3-V_HCD3-V_HCEA-V_LCEA.

IV. Antibody Preparation

[0219] Antibodies described herein are prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

[0220] The antibody is directed against an antigen of interest (*i.e.*, PD-L1 (such as a human PD-L1), CEA, or CD3 (such as a human CD3)). Preferably, the antigen is a biologically important polypeptide and administration of the antibody to a mammal suffering from a disorder can result in a therapeutic benefit in that mammal.

[0221] In certain embodiments, an antibody provided herein has a dissociation constant (K_D) of $\leq 1\mu M$, ≤ 150 nM, ≤ 100 nM, ≤ 50 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, *e.g.,* from 10^{-9} M to 10^{-13} M).

[0222] In one embodiment, K_D is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.,* Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest. The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period *(e.g.,* about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature *(e.g.,* for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0223] According to another embodiment, K_D is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at -10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (-0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, *e.g.,* Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(i) Antigen Preparation

[0224] Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (e.g. the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (e.g. cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule. Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

(ii) Certain Antibody-Based Methods

[0225] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[0226] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0227] Monoclonal antibodies can be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), and further described, e.g., in Hongo et al., Hybridoma, 14 (3): 253-260 (1995), Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981), and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) regarding human-human hybridomas. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 re-garding production of monoclonal human natural IgM antibodies from hybridoma cell lines. Human hybridoma technology (Trioma technology) is described in Vollmers and Brandlein, Histology and Histopathology, 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Experimental and Clinical Pharmacology, 27(3):185-91 (2005).

[0228] For various other hybridoma techniques, see, *e.g.,* US 2006/258841; US 2006/183887 (fully human antibodies), US 2006/059575; US 2005/287149; US 2005/100546; US 2005/026229; and U.S. Pat. Nos. 7,078,492 and 7,153,507. An exemplary protocol for producing monoclonal antibodies using the hybridoma method is described as follows. In one embodiment, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of a polypeptide of the invention or a fragment thereof, and an adjuvant, such as monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, Mont.). A polypeptide of the invention (e.g., antigen) or a fragment thereof may be prepared using methods well known in the art, such as recombinant methods, some of which are further described herein. Serum from immunized animals is assayed for anti-antigen antibodies, and booster immunizations are optionally administered. Lymphocytes from animals producing anti-antigen antibodies are isolated. Alternatively, lymphocytes may be immunized *in vitro.*

[0229] Lymphocytes are then fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. See, *e.g.,* Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986). Myeloma cells may be used that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0230] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium, e.g., a medium that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells. Preferably, serum-free hybridoma cell culture methods are used to reduce use of animalderived serum such as fetal bovine serum, as described, for example, in Even et al., Trends in Biotechnology, 24(3), 105-108 (2006).

[0231] Oligopeptides as tools for improving productivity of hybridoma cell cultures are described in Franek, Trends in Monoclonal Antibody Research, 111-122 (2005). Specifically, standard culture media are enriched with certain amino acids (alanine, serine, asparagine, proline), or with protein hydrolyzate fractions, and apoptosis may be significantly suppressed by synthetic oligopeptides, constituted of three to six amino acid residues. The peptides are present at millimolar or higher concentrations.

[0232] Culture medium in which hybridoma cells are growing may be assayed for production of monoclonal antibodies that bind to an antibody of the invention. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbent assay (ELISA). The binding affinity of the monoclonal antibody can be determined, for example, by Scatchard analysis. See, *e.g.,* Munson et al., Anal. Biochem., 107:220 (1980).

[0233] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods. See, *e.g.,* Goding, *supra.* Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, hybridoma cells may be grown *in vivo* as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. One procedure for isolation of proteins from hybridoma cells is described in US 2005/176122 and U.S. Pat. No. 6,919,436. The method includes using minimal salts, such as lyotropic salts, in the binding process and preferably also using small amounts of organic solvents in the elution process.

(iii) Library-Derived Antibodies

[0234] Antibodies useful in the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Additional methods are reviewed, *e.g.,* in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.,* in the McCafferty et al., Nature 348:552-554; Clackson et al., Nature 352: 624-628 (1991); Marks et al., J. Mol. Biol. 222: 581-597 (1992); Marks and Bradbury, in Methods in Molecular Biology 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., J. Mol. Biol. 338(2): 299-310 (2004); Lee et al., J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al., J. Immunol. Methods 284(1-2): 119-132(2004).

[0235] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., Ann. Rev. Immunol., 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned *(e.g.,* from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro,* as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0236] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

(iv) Chimeric, Humanized and Human Antibodies

[0237] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.,* in U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.,* a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0238] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.,* the antibody from which the HVR residues are derived), *e.g.,* to restore or improve antibody specificity or affinity.

[0239] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front. Biosci.

13:1619-1633 (2008), and are further described, *e.g.,* in Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., Methods 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., Methods 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36:61-68 (2005) and Klimka et al., Br. J. Cancer, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0240] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.,* Sims et al. J. Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.,* Carter et al. Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta et al. J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.,* Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.,* Baca et al., J. Biol. Chem. 272:10678-10684 (1997) and Rosok et al., J. Biol. Chem. 271:22611-22618 (1996)).

[0241] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0242] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). See also, *e.g.,* U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.,* by combining with a different human constant region.

[0243] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.,* Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of mon-oclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Bran-dlein, Histology and Histopathology, 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Ex-perimental and Clinical Pharmacology, 27(3):185-91 (2005).

[0244] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

(v) Antibody Fragments

[0245] Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, and may lead to improved access to solid tumors. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

[0246] Various techniques have been developed for the production of antibody fragments. Traditionally, these frag-ments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894;

and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. See Antibody Engineering, ed. Borrebaeck, *supra*. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

(vi) Multispecific Antibodies

[0247] Multispecific antibodies have binding specificities for at least two different epitopes, where the epitopes are usually from different antigens. While such molecules normally will only bind two different epitopes (i.e. bispecific antibodies, BsAbs), antibodies with additional specificities such as trispecific antibodies are encompassed by this expression when used herein. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')$_2$ bispecific antibodies).

[0248] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

[0249] One approach known in the art for making bispecific antibodies is the "knobs-into-holes" or "protuberance-into-cavity" approach (see, *e.g.,* US Pat. No. 5,731,168). In this approach, two immunoglobulin polypeptides (e.g., heavy chain polypeptides) each comprise an interface. An interface of one immunoglobulin polypeptide interacts with a corresponding interface on the other immunoglobulin polypeptide, thereby allowing the two immunoglobulin polypeptides to associate. These interfaces may be engineered such that a "knob" or "protuberance" (these terms may be used interchangeably herein) located in the interface of one immunoglobulin polypeptide corresponds with a "hole" or "cavity" (these terms may be used interchangeably herein) located in the interface of the other immunoglobulin polypeptide. In some embodiments, the hole is of identical or similar size to the knob and suitably positioned such that when the two interfaces interact, the knob of one interface is positionable in the corresponding hole of the other interface. Without wishing to be bound to theory, this is thought to stabilize the heteromultimer and favor formation of the heteromultimer over other species, for example homomultimers. In some embodiments, this approach may be used to promote the heteromultimerization of two different immunoglobulin polypeptides, creating a bispecific antibody comprising two immunoglobulin polypeptides with binding specificities for different epitopes.

[0250] In some embodiments, a knob may be constructed by replacing a small amino acid side chain with a larger side chain. In some embodiments, a hole may be constructed by replacing a large amino acid side chain with a smaller side chain. Knobs or holes may exist in the original interface, or they may be introduced synthetically. For example, knobs or holes may be introduced synthetically by altering the nucleic acid sequence encoding the interface to replace at least one "original" amino acid residue with at least one "import" amino acid residue. Methods for altering nucleic acid sequences may include standard molecular biology techniques well known in the art. The side chain volumes of various amino acid residues are shown in the following table. In some embodiments, original residues have a small side chain volume (e.g., alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine), and import residues for forming a knob are naturally occurring amino acids and may include arginine, phenylalanine, tyrosine, and tryptophan. In some embodiments, original residues have a large side chain volume (*e.g.*, arginine, phenylalanine, tyrosine, and tryptophan), and import residues for forming a hole are naturally occurring amino acids and may include alanine, serine, threonine, and valine.

Table 1. Properties of amino acid residues

Amino acid	One-letter abbreviation	Mass[a] (daltons)	Volume[b] (Å^3)	Accessible surface area[c] (Å^2)
Alanine (Ala)	A	71.08	88.6	115
Arginine (Arg)	R	156.20	173.4	225
Asparagine (Asn)	N	114.11	117.7	160
Aspartic Acid (Asp)	D	115.09	111.1	150
Cysteine (Cys)	c	103.14	108.5	135
Glutamine (Gln)	Q	128.14	143.9	180

(continued)

Amino acid	One-letter abbreviation	Mass[a] (daltons)	Volume[b] (Å^3)	Accessible surface area[c] (Å^2)
Glutamic Acid (Glu)	E	129.12	138.4	190
Glycine (Gly)	G	57.06	60.1	75
Histidine (His)	H	137.15	153.2	195
Isoleucine (Ile)	I	113.17	166.7	175
Leucine (Leu)	L	113.17	166.7	170
Lysine (Lys)	K	128.18	168.6	200
Methionine (Met)	M	131.21	162.9	185
Phenylalanine (Phe)	F	147.18	189.9	210
Proline (Pro)	P	97.12	122.7	145
Serine (Ser)	S	87.08	89.0	115
Threonine (Thr)	T	101.11	116.1	140
Tryptophan (Trp)	w	186.21	227.8	255
Tyrosine (Tyr)	Y	163.18	193.6	230
Valine (Val)	V	99.14	140.0	155

[a]Molecular weight of amino acid minus that of water. Values from Handbook of Chemistry and Physics, 43rd ed. Cleveland, Chemical Rubber Publishing Co., 1961.
[b]Values from A.A. Zamyatnin, Prog. Biophys. Mol. Biol. 24:107-123, 1972.
[c]Values from C. Chothia, J. Mol. Biol. 105:1-14, 1975. The accessible surface area is defined in Figures 6-20 of this reference.

[0251] In some embodiments, original residues for forming a knob or hole are identified based on the three-dimensional structure of the heteromultimer. Techniques known in the art for obtaining a three-dimensional structure may include X-ray crystallography and NMR. In some embodiments, the interface is the CH3 domain of an immunoglobulin constant domain. In these embodiments, the CH3/CH3 interface of human IgG$_1$ involves sixteen residues on each domain located on four anti-parallel β-strands. Without wishing to be bound to theory, mutated residues are preferably located on the two central anti-parallel β-strands to minimize the risk that knobs can be accommodated by the surrounding solvent, rather than the compensatory holes in the partner CH3 domain. In some embodiments, the mutations forming corresponding knobs and holes in two immunoglobulin polypeptides correspond to one or more pairs provided in the following table.

Table 2. Exemplary sets of corresponding knob-and hole-forming mutations

CH3 of first immunoglobulin	CH3 of second immunoglobulin
T366Y	Y407T
T366W	Y407A
F405A	T394W
Y407T	T366Y
T366Y:F405A	T394W:Y407T
T366W:F405W	T394S:Y407A
F405W:Y407A	T366W:T394S
F405W	T394S

Mutations are denoted by the original residue, followed by the position using the EU numbering system, and then the import residue (all residues are given in single-letter amino acid code). Multiple mutations are separated by a colon.

[0252] In some embodiments, an immunoglobulin polypeptide comprises a CH3 domain comprising one or more amino acid substitutions listed in Table 2 above. In some embodiments, a bispecific antibody comprises a first immunoglobulin polypeptide comprising a CH3 domain comprising one or more amino acid substitutions listed in the left column of Table 2, and a second immunoglobulin polypeptide comprising a CH3 domain comprising one or more corresponding amino acid substitutions listed in the right column of Table 2.

[0253] Following mutation of the DNA as discussed above, polynucleotides encoding modified immunoglobulin polypeptides with one or more corresponding knob- or hole-forming mutations may be expressed and purified using standard recombinant techniques and cell systems known in the art. See, e.g., U.S. Pat. Nos. 5,731,168; 5,807,706; 5,821,333; 7,642,228; 7,695,936; 8,216,805; U.S. Pub. No. 2013/0089553; and Spiess et al., Nature Biotechnology 31: 753-758, 2013. Modified immunoglobulin polypeptides may be produced using prokaryotic host cells, such as *E. coli,* or eukaryotic host cells, such as CHO cells. Corresponding knob- and hole-bearing immunoglobulin polypeptides may be expressed in host cells in co-culture and purified together as a heteromultimer, or they may be expressed in single cultures, separately purified, and assembled *in vitro.* In some embodiments, two strains of bacterial host cells (one expressing an immunoglobulin polypeptide with a knob, and the other expressing an immunoglobulin polypeptide with a hole) are co-cultured using standard bacterial culturing techniques known in the art. In some embodiments, the two strains may be mixed in a specific ratio, *e.g.,* so as to achieve equal expression levels in culture. In some embodiments, the two strains may be mixed in a 50:50, 60:40, or 70:30 ratio. After polypeptide expression, the cells may be lysed together, and protein may be extracted. Standard techniques known in the art that allow for measuring the abundance of homo-multimeric vs. hetero-multimeric species may include size exclusion chromatography. In some embodiments, each modified immunoglobulin polypeptide is expressed separately using standard recombinant techniques, and they may be assembled together *in vitro.* Assembly may be achieved, for example, by purifying each modified immunoglobulin polypeptide, mixing and incubating them together in equal mass, reducing disulfides (*e.g.,* by treating with dithiothreitol), concentrating, and reoxidizing the polypeptides. Formed bispecific antibodies may be purified using standard techniques including cation-exchange chromatography and measured using standard techniques including size exclusion chromatography. For a more detailed description of these methods, see Spiess et al., Nat Biotechnol 31:753-8, 2013. In some embodiments, modified immunoglobulin polypeptides may be expressed separately in CHO cells and assembled *in vitro* using the methods described above.

[0254] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is typical to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0255] In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

[0256] According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. One interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0257] Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

[0258] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')$_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0259] Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')$_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody.

[0260] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a lightchain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al, J. Immunol, 152:5368 (1994).

[0261] Another technique for making bispecific antibody fragments is the "bispecific T cell engager" or BiTE® approach (see, *e.g.,* WO2004/106381, WO2005/061547, WO2007/042261, and WO2008/119567). This approach utilizes two antibody variable domains arranged on a single polypeptide. For example, a single polypeptide chain includes two single chain Fv (scFv) fragments, each having a variable heavy chain (V_H) and a variable light chain (V_L) domain separated by a polypeptide linker of a length sufficient to allow intramolecular association between the two domains. This single polypeptide further includes a polypeptide spacer sequence between the two scFv fragments. Each scFv recognizes a different epitope, and these epitopes may be specific for different cell types, such that cells of two different cell types are brought into close proximity or tethered when each scFv is engaged with its cognate epitope. One particular embodiment of this approach includes a scFv recognizing a cell-surface antigen expressed by an immune cell, *e.g.,* a CD3 polypeptide on a T cell, linked to another scFv that recognizes a cell-surface antigen expressed by a target cell, such as a malignant or tumor cell.

[0262] As it is a single polypeptide, the bispecific T cell engager may be expressed using any prokaryotic or eukaryotic cell expression system known in the art, *e.g.,* a CHO cell line. However, specific purification techniques (see, *e.g.,* EP1691833) may be necessary to separate monomeric bispecific T cell engagers from other multimeric species, which may have biological activities other than the intended activity of the monomer. In one exemplary purification scheme, a solution containing secreted polypeptides is first subjected to a metal affinity chromatography, and polypeptides are eluted with a gradient of imidazole concentrations. This eluate is further purified using anion exchange chromatography, and polypeptides are eluted using with a gradient of sodium chloride concentrations. Finally, this eluate is subjected to size exclusion chromatography to separate monomers from multimeric species.

[0263] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tuft et al. J. Immunol. 147: 60 (1991).

(vii) Single-Domain Antibodies

[0264] A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; *see, e.g.,* U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

(viii) Antibody Variants

[0265] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide

sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

(ix) Substitution, Insertion, and Deletion Variants

[0266] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 3. Exemplary Substitutions.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0267] Amino acids may be grouped according to common side-chain properties:

    a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

    b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

    c. acidic: Asp, Glu;

    d. basic: His, Lys, Arg;

e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[0268] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0269] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0270] Alterations (*e.g.,* substitutions) may be made in HVRs, *e.g.,* to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.,* residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.,* Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.,* in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.,* 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0271] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0272] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (*e.g.,* charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.,* alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0273] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.,* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

(x) Glycosylation variants

[0274] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0275] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.,* Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.,* mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0276] In one embodiment, antibody variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.,* a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, the antibody is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In certain embodiments, the antibody is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.,* wherein the antibody is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about $\pm$ 3 amino acids upstream or downstream of position 297, *i.e.,* between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, *e.g.,* Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).

[0277] Antibody variants are further provided with bisected oligosaccharides, *e.g.,* in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.,* in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); US 2005/0123546 (Umana et al.), and Ferrara et al., Biotechnology and Bioengineering, 93(5): 851-861 (2006). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.,* in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0278] In certain embodiments, the antibody variants comprising an Fc region described herein are capable of binding to an FcγRIII. In certain embodiments, the antibody variants comprising an Fc region described herein have ADCC activity in the presence of human effector cells or have increased ADCC activity in the presence of human effector cells compared to the otherwise same antibody comprising a human wild-type IgGlFc region.

(xi) Fc region variants

[0279] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.,* a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0280] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector

cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.,* in an animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.,* C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M.S. et al., Blood 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.,* Petkova, S.B. et al., Int'l. Immunol. 18(12):1759-1769 (2006)).

[0281] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0282] Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).)

[0283] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In an exemplary embodiment, the antibody comprising the following amino acid substitutions in its Fc region: S298A, E333A, and K334A.

[0284] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.,* either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.,* as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[0285] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.)). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

(xii) Antibody Derivatives

[0286] The antibodies used in the invention can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. In certain embodiments, the moieties suitable for derivatization of the antibody are water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

(xiii) Vectors, Host Cells, and Recombinant Methods

[0287] Antibodies may also be produced using recombinant methods. For recombinant production of an anti-antigen antibody, nucleic acid encoding the antibody is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody may be readily isolated and sequenced using conventional procedures (*e.g.,* by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(a) Signal Sequence Component

[0288] An antibody may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (e.g., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process a native antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.,* the yeast invertase leader, a factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

(b) Origin of Replication

[0289] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter.

(c) Selection Gene Component

[0290] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.,* the gene encoding D-alanine racemase for *Bacilli.*
[0291] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.
[0292] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up antibody-encoding nucleic acid, such as DHFR, glutamine synthetase (GS), thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*
[0293] For example, cells transformed with the DHFR gene are identified by culturing the transformants in a culture medium containing methotrexate (Mtx), a competitive antagonist of DHFR. Under these conditions, the DHFR gene is amplified along with any other co-transformed nucleic acid. A Chinese hamster ovary (CHO) cell line deficient in endogenous DHFR activity (e.g., ATCC CRL-9096) may be used.
[0294] Alternatively, cells transformed with the GS gene are identified by culturing the transformants in a culture medium containing L-methionine sulfoximine (Msx), an inhibitor of GS. Under these conditions, the GS gene is amplified along with any other co-transformed nucleic acid. The GS selection/amplification system may be used in combination with the DHFR selection/amplification system described above.
[0295] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody of interest, wild-type DHFR gene, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.
[0296] A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb et al., Nature, 282:39 (1979)). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85:12 (1977). The presence of the *trp*1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu*2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu*2 gene.
[0297] In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis.* Van den Berg, Bio/Technology, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature

recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al., Bio/Technology, 9:968-975 (1991).

(d) Promoter Component

[0298] Expression and cloning vectors generally contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding an antibody. Promoters suitable for use with prokaryotic hosts include the *phoA* promoter, β-lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding an antibody.

[0299] Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

[0300] Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0301] Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

[0302] Antibody transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, Simian Virus 40 (SV40), or from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0303] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

(e) Enhancer Element Component

[0304] Transcription of a DNA encoding an antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

(f) Transcription Termination Component

[0305] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(g) Selection and Transformation of Host Cells

[0306] Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g., B. licheniformis* 41P disclosed in DD 266,710 published 12 Apr. 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

[0307] Full length antibody, antibody fusion proteins, and antibody fragments can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (e.g., a toxin) that by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half-life in circulation. Production in E. coli is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Pat. No. 5,648,237 (Carter et. al.), U.S. Pat. No. 5,789,199 (Joly et al.), U.S. Pat. No. 5,840,523 (Simmons et al.), which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion. *See also* Charlton, Methods in Molecular Biology, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 245-254, describing expression of antibody fragments in *E. coli.* After expression, the antibody may be isolated from the E. coli cell paste in a soluble fraction and can be purified through, e.g., a protein A or G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed e.g., in CHO cells.

[0308] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors. *Saccharomyces cerevisiae,* or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe; Kluyveromyces* hosts such as, *e.g., K. lactis, K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans,* and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa; Schwanniomyces* such as *Schwanniomyces occidentalis;* and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium,* and *Aspergillus* hosts such as *A. nidulans* and *A. niger.* For a review discussing the use of yeasts and filamentous fungi for the production of therapeutic proteins, see, e.g., Gerngross, Nat. Biotech. 22:1409-1414 (2004).

[0309] Certain fungi and yeast strains may be selected in which glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See, e.g.,* Li et al., Nat. Biotech. 24:210-215 (2006) (describing humanization of the glycosylation pathway in *Pichia pastoris);* and Gerngross et al., *supra.*

[0310] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.,* the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the invention, particularly for transfection of *Spodoptera frugiperda* cells.

[0311] Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, duckweed (*Leninaceae*), alfalfa (*M. truncatula*)*,* and tobacco can also be utilized as hosts. *See, e.g.,* U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0312] Vertebrate cells may be used as hosts, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR⁻ CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268.

[0313] Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(h) Culturing the Host Cells

[0314] The host cells used to produce an antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), anti-biotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

(xiv) Purification of Antibody

[0315] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli.* Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0316] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being among one of the typically preferred purification steps. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0317] In general, various methodologies for preparing antibodies for use in research, testing, and clinical are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

C. Selecting Biologically Active Antibodies

[0318] Antibodies produced as described above may be subjected to one or more "biological activity" assays to select an antibody with beneficial properties from a therapeutic perspective or selecting formulations and conditions that retain biological activity of the antibody. The antibody may be tested for its ability to bind the antigen against which it was raised. For example, methods known in the art (such as ELISA, Western Blot, etc.) may be used.

[0319] For example, for an anti-PD-LI antibody, the antigen binding properties of the antibody can be evaluated in an assay that detects the ability to bind to PD-L1. In some embodiments, the binding of the antibody may be determined

by saturation binding; ELISA; and/or competition assays (e.g. RIA's), for example. Also, the antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. For example, the biological effects of PD-L1 blockade by the antibody can be assessed in CD8+T cells, a lymphocytic choriomeningitis virus (LCMV) mouse model and/or a syngeneic tumor model e.g., as described in US Patent 8,217,149.

[0320] To screen for antibodies which bind to a particular epitope on the antigen of interest (e.g., those which block binding of the anti-PD-LI antibody of the example to PD-L1), a routine cross-blocking assay such as that described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, e.g. as described in Champe et al., J. Biol. Chem. 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

D. Pharmaceutical Compositions and Formulations

[0321] Also provided herein are pharmaceutical compositions and formulations comprising a PD-1 axis binding antagonist and/or an antibody described herein (such as an anti-PD-L1 antibody, or a bispecific antibody that binds CEA and CD3) and a pharmaceutically acceptable carrier.

[0322] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (e.g. a PD-1 axis binding antagonist and/or an anti-CEA/anti-CD3 bispecific antibody) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include intersterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0323] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0324] The composition and formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0325] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0326] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

IV. Methods of Treatment

[0327] Provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. In some embodiments, the treatment results in a response in the individual after treatment. In some embodiments, the response is a partial response. In some embodiments, the response is a complete response. In some embodiments, the treatment results in a sustained response (e.g., a sustained partial response or complete response)

in the individual after cessation of the treatment. The methods described herein may find use in treating conditions where enhanced immunogenicity is desired such as increasing tumor immunogenicity for the treatment of cancer. Also provided herein are methods of enhancing immune function in an individual having cancer comprising administering to the individual an effective amount of a PD-1 axis binding antagonist (e.g., MPDL3280A) and an anti-CEA/anti-CD3 bispecific antibody (e.g. CEA TCB).

[0328]   In some instances, the methods provided herein include administration of an effective amount of a PD-1 axis binding antagonist selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist, and a PD-L2 binding antagonist. In some instances, the PD-L1 binding antagonist is an antibody, such as an antibody that is capable of inhibiting PD-L1 binding to PD-1 and B7.1, but does not disrupt binding of PD-1 to PD-L2. In some instances, the PD-L1 binding antagonist antibody is MPDL3280A, which may be administered at a dose of about 800 mg to about 1500 mg every three weeks (e.g., about 1000 mg to about 1300 mg every three weeks, e.g., about 1100 mg to about 1200 mg every three weeks). In some embodiments, MPDL3280A is administered at a dose of about 1200 mg every three weeks.

[0329]   As a general proposition, the therapeutically effective amount of a PD-1 axis binding antagonist (e.g., an anti-PD-Ll antibody, e.g., MPDL3280A) may be administered to a human will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, for example, the antagonist (e.g., anti-PD-Ll antibody, e.g., MPDL3280A) is administered in a dose of about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, a PD-1 axis binding antagonist (e.g., anti-PD-L1 antibody, e.g., MPDL3280A) is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, or about 1500 mg. In some embodiments, a PD-1 axis binding antagonist (e.g., anti-PD-Ll antibody, e.g., MPDL3280A) is administered at a dose of about 1150 mg to about 1250 mg every three weeks. In some embodiments, a PD-1 axis binding antagonist (e.g., anti-PD-Ll antibody, e.g., MPDL3280A) is administered at a dose of about 1200 mg every three weeks. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. In some embodiments, for example, the method for treating or delaying progression of cancer in an individual comprises a dosing regimen comprising treatment cycles, wherein the individual is administered, on days 1 of each cycle, a human PD-1 axis binding antagonist (e.g., anti-PD-Ll antibody, e.g., MPDL3280A) at a dose of about 1200 mg, wherein each cycle is 21 days (i.e., each cycle is repeated every 21 days). The progress of this therapy is easily monitored by conventional techniques.

[0330]   In some instances, the methods provided herein include administration of an effective amount of an anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB). In some instances, the anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) is administered to the individual at a dose of about 5 mg to about 400 mg every week (e.g., about 10 mg to about 60 mg every week, e.g., about 10 mg to about 40 mg every week, e.g. about 80 mg to about 200 mg every week, e.g. about 80 mg to about 400 mg every week, or e.g. about 160 mg to 400 mg every week). In some embodiments, the anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) is administered at a dose of about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg or about 160 mg, every week. As a general proposition, the therapeutically effective amount of an anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) administered to a human will be in the range of about 5 to about 400 mg (e.g., about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 150 mg, about 160 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, or about 400 mg), whether by one or more administrations. For example, in some embodiments, about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg or about 160 mg, of CEA TCB is administered. In some embodiments, CEA TCB is administered at 5 mg, 10 mg, 20 mg, 40 mg, 80 mg or 160 mg, once a week. In particular embodiments, at least about 80 mg of CEA TCB is administered. In some embodiments, CEA TCB is administered at 80 mg once a week. In some embodiments, CEA TCB is administered at 80-200 mg, 80-400 mg or 160-400 mg once a week. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) may be administered weekly, every 2 weeks, every 3 weeks, every 4 weeks, on days 1, 8 and 15 of each 21-day cycle, or on days 1, 8, and 15 of each 28-day cycle.

[0331]   In some instances, the PD-1 axis binding antagonist (e.g., an anti-PD-Ll antibody, e.g., MPDL3280A) and the anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) are administered in a single dosing regimen. The administration of these agents may be concurrent or separate within the context of the dosing regimen. For example, in some instances, the methods provided herein include a dosing regimen comprising treatment cycles, wherein the individual is administered, on days 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8,

and 15 of each cycle, an anti-CEA/anti-CD3 bispecific antibody at a dose of about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg or about 160 mg, each cycle being repeated every 21 days. In one embodiment, the individual is administered, on days 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle, an anti-CEA/anti-CD3 bispecific antibody at a dose of about 80 mg, each cycle being repeated every 21 days. In a particular embodiment, the individual is administered, on days 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle, an anti-CEA/anti-CD3 bispecific antibody at a dose of at least about 80 mg, each cycle being repeated every 21 days. In still a further embodiment, the individual is administered, on days 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle, an anti-CEA/anti-CD3 bispecific antibody at a dose of about 80 mg to about 200 mg. about 80 mg to about 400 mg, or about 160 mg to about 400 mg, each cycle being repeated every 21 days.

[0332] In some embodiments, the individual is a human. In some embodiments, the individual is suffering from locally advanced or metastatic breast cancer. In some embodiments, the individual has CEA positive cancer. In some embodiments, CEA positive cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer. In a particular embodiment, the cancer is colorectal carcinoma. In some embodiments, the breast cancer is a breast carcinoma or a breast adenocarcinoma. In some embodiments, the breast carcinoma is an invasive ductal carcinoma. In some embodiments, the lung cancer is a lung adenocarcinoma. In some embodiments, the colon cancer is a colorectal adenocarcinoma. In some embodiments, the cancer cells in the individual express PD-L1. In some embodiments, the cancer cells in the individual express CEA protein at a level that is detectable (e.g., detectable using methods known in the art).

[0333] In some embodiments, the individual has been treated with a cancer therapy before the combination treatment with a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. In some embodiments, the individual has cancer that is resistant to one or more cancer therapies. In some embodiments, resistance to cancer therapy includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to a cancer therapy includes progression of the cancer during treatment with the anti-cancer therapy. In some embodiments, resistance to a cancer therapy includes cancer that does not response to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment. In some embodiments, the cancer is at early stage or at late stage.

[0334] In some embodiments, the combination therapy of the invention comprises administration of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody. The PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody may be administered in any suitable manner known in the art. For example, The PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the PD-1 axis binding antagonist is in a separate composition as the anti-CEA/anti-CD3 bispecific antibody. In some embodiments, the PD-1 axis binding antagonist is in the same composition as the anti-CEA/anti-CD3 bispecific antibody.

[0335] The PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the anti-CEA/anti-CD3 bispecific antibody is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. An effective amount of the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody may be administered for prevention or treatment of disease. The appropriate dosage of the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody may be determined based on the type of disease to be treated, the type of the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0336] In some embodiments, the methods may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (e.g., lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents described herein.

Other Combination Therapies

[0337] Also provided herein are methods for treating or delaying progression of cancer in an individual comprising administering to the individual a human PD-1 axis binding antagonist (e.g., anti-PD-LI antibody, e.g., MPDL3280A) and an anti-CEA/anti-CD3 bispecific antibody (e.g., CEA TCB) in conjunction with another anti-cancer agent or cancer therapy.

[0338] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a chemotherapy or chemotherapeutic agent. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a radiation therapy or radiotherapeutic agent. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a targeted therapy or targeted therapeutic agent. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an immunotherapy or immunotherapeutic agent, for example a monoclonal antibody.

[0339] Without wishing to be bound to theory, it is thought that enhancing T cell stimulation, by promoting an activating co-stimulatory molecule or by inhibiting a negative co-stimulatory molecule, may promote tumor cell death thereby treating or delaying progression of cancer. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agonist directed against an activating co-stimulatory molecule. In some embodiments, an activating co-stimulatory molecule may include CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, the agonist directed against an activating co-stimulatory molecule is an agonist antibody that binds to CD40, CD226, CD28, OX40, GITR, CD137, CD27, HVEM, or CD127. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antagonist directed against an inhibitory co-stimulatory molecule. In some embodiments, an inhibitory co-stimulatory molecule may include CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase. In some embodiments, the antagonist directed against an inhibitory co-stimulatory molecule is an antagonist antibody that binds to CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO, TIGIT, MICA/B, or arginase.

[0340] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antagonist directed against CTLA-4 (also known as CD152), *e.g.,* a blocking antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with ipilimumab (also known as MDX-010, MDX-101, or YERVOY®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with tremelimumab (also known as ticilimumab or CP-675,206). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antagonist directed against B7-H3 (also known as CD276), *e.g.,* a blocking antibody. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with MGA271. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antagonist directed against a TGF beta, *e.g.,* metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299.

[0341] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment comprising adoptive transfer of a T cell *(e.g.,* a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment comprising adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, *e.g,* a dominant-negative TGF beta type II receptor. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment comprising a HERCREEM protocol (see, e.g., ClinicalTrials.gov Identifier NCT00889954).

[0342] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with urelumab (also known as BMS-663513). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agonist directed against CD40, *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with CP-870893. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agonist directed against OX40 (also known as CD134), *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an anti-OX40 antibody (e.g., AgonOX). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agonist directed against CD27, *e.g.,* an activating antibody. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with CDX-1127. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antagonist directed against indoleamine-2,3-dioxygenase (IDO). In some embodiments, with the IDO antagonist is

1-methyl-D-tryptophan (also known as 1-D-MT).

[0343] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antibody-drug conjugate. In some embodiments, the antibody-drug conjugate comprises mertansine or monomethyl auristatin E (MMAE). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with and anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A or RG7599). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with DMUC5754A. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), *e.g.,* an antibody directed against EDNBR conjugated with MMAE.

[0344] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an angiogenesis inhibitor. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an antibody directed against a VEGF, *e.g.,* VEGF-A. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with bevacizumab (also known as AVASTIN®, Genentech). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antibody directed against angiopoietin 2 (also known as Ang2). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with MEDI3617.

[0345] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antineoplastic agent. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an agent targeting CSF-1R (also known as M-CSFR or CD115). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with anti-CSF-IR (also known as IMC-CS4). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an interferon, for example interferon alpha or interferon gamma. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with Roferon-A (also known as recombinant Interferon alpha-2a). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or Leukine®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with IL-2 (also known as aldesleukin or Proleukin®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with IL-12. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antibody targeting CD20. In some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or Gazyva®) or rituximab. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an antibody targeting GITR. In some embodiments, the antibody targeting GITR is TRX518.

[0346] In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a cancer vaccine. In some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine. In some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., Cancer Sci, 104:14-21, 2013). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an adjuvant. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment comprising a TLR agonist, *e.g.,* Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with tumor necrosis factor (TNF) alpha. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with IL-1. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with HMGB1. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an IL-10 antagonist. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an IL-4 antagonist. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an IL-13 antagonist. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an HVEM antagonist. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an ICOS agonist, *e.g.,* by administration of ICOS-L, or an agonistic antibody directed against ICOS. In some embodiments, a PD-1 axis binding antagonist and

an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment targeting CX3CL1. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment targeting CXCL9. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment targeting CXCL10. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a treatment targeting CCL5. In some embodiments, a PD-1 axis binding antagonist may be administered in conjunction with an LFA-1 or ICAM1 agonist. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a Selectin agonist.

[0347]   In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a targeted therapy. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of B-Raf. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with vemurafenib (also known as Zelboraf®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with dabrafenib (also known as Tafinlar®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with erlotinib (also known as Tarceva®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) or MEK2 (also known as MAP2K2). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with cobimetinib (also known as GDC-0973 or XL-518). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with trametinib (also known as Mekinist®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of K-Ras. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of c-Met. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with onartuzumab (also known as MetMAb). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of Alk. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with AF802 (also known as CH5424802 or alectinib). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of a phosphatidylinositol 3-kinase (PI3K). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with BKM120. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with idelalisib (also known as GS-1101 or CAL-101). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with perifosine (also known as KRX-0401). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of an Akt. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with MK2206. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with GSK690693. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with GDC-0941. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with an inhibitor of mTOR. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with sirolimus (also known as rapamycin). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with temsirolimus (also known as CCI-779 or Torisel®). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with everolimus (also known as RAD001). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with ridaforolimus (also known as AP-23573, MK-8669, or deforolimus). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with OSI-027. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with AZD8055. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with INK128. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with a dual PI3K/mTOR inhibitor. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with XL765. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with GDC-0980. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with BEZ235 (also known as NVP-BEZ235). In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with BGT226. In some

embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with GSK2126458. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with PF-04691502. In some embodiments, a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody may be administered in conjunction with PF-05212384 (also known as PKI-587).

V. Articles of Manufacture or Kits

[0348] In another embodiment of the invention, an article of manufacture or a kit is provided comprising a PD-1 axis binding antagonist and/or an anti-CEA/anti-CD3 bispecific antibody. In some embodiments, the article of manufacture or kit further comprises package insert comprising instructions for suing the PD-1 axis binding antagonist in conjunction with an anti-CEA/anti-CD3 bispecific antibody to treat or delay progression of cancer in an individual or to enhance immune function of an individual having cancer. Any of the PD-1 axis binding antagonist and/or an anti-CEA/anti-CD3 bispecific antibodies described herein may be included in the article of manufacture or kits.

[0349] In some embodiments, the PD-1 axis binding antagonist and the anti-CEA/anti-CD3 bispecific antibody are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. In some embodiments, the article of manufacture further includes one or more of another agent (e.g., a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0350] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0351] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: CEA TCB-mediated tumor lysis leads to up-regulation of PD-1 on human T cells and of PD-L1 on surviving tumor cells in vitro

[0352] CEA TCB is a novel T cell bispecific antibody targeting the carcinoembryonic antigen (CEA) on tumor cells and CD3 on T cells. It is currently being investigated as a single agent in a Phase I study in patients with advanced and /or metastatic CEA expressing tumors.

[0353] The lysis of CEA-expressing MKN-45 target cells mediated by CEA TCB was assessed after 24 h and 48 h of incubation with human PBMCs (E:T 10:1, LDH release) (Figure 1, A 24 h, B 48 h).

[0354] PBMCs were isolated from a buffy coat obtained from the "Blutspende Zürich". Blood was diluted 3:1 with PBS. About 30 ml of the blood/PBS mixture was layered on 15 ml of Histopaque and centrifuged for 30 min at 450 g without brake at room temperature (RT). Lymphocytes were collected with a 10 ml pipette into 50 ml tubes containing PBS and filled up to 50 ml with PBS and centrifuged 10 min at 350 g. The supernatant was discarded, the pellet re-suspended in 50 ml PBS and centrifuged for 10 min at 300 g. The washing step was repeated once. Cells were re-suspended in RPMI containing 10% FCS and 1% GlutaMax and stored at 37°C, 5% CO_2 in the incubator until the initiation of the assay.

[0355] Adherent MKN-45 target cells were harvested with Trypsin/EDTA one day before the assay, washed and plated at density of 1.4 mio cells/well using flat-bottom 24-well plates. Cells were left to adhere overnight in a humidified incubator. On the day of the assay, plates were centrifuged at 350 g for 5 min and the medium was aspirated. 550 µl per well of assay medium (RPMI1640, 2% FCS, 1% GlutaMax) were added.

[0356] CEA TCB or untargeted TCB were added at indicated concentrations (range of 6.4 pM - 100 nM, in triplicates). PBMCs were added to target cells at the final Effector:Target ratio E:T 10:1, final volume per well was 1.1 ml.

[0357] Target cell killing was assessed after 24 h and 48 h of incubation by quantification of LDH (lactate dehydrogenase) released into cell supernatants by apoptotic/necrotic cells (LDH detection kit, Roche Applied Science, #11 644

793 001). Maximal lysis of the target cells (= 100%) was achieved by incubation of target cells with 1% Triton X-100. Minimal lysis (= 0%) refers to target cells co-incubated with effector cells without bispecific antibody. The EC50 values were calculated using GraphPadPrism5.

[0358] Following killing, the surface expression of PD-1 receptor (on CD4+ or CD8+ T cells) and of PD-L1 (on tumor cells that survived killing) was assessed by flow cytometry.

[0359] Upon removal of 50 μl of the supernatant for the second LDH read-out (48h time point), the assay plates were centrifuged for 5 min at 350 g and the supernatants were discarded. Cell pellets were re-suspended in 100 μl PBS and transferred into wells of a 96-round-bottom well plate. The wells were washed with 80 μl PBS and this washing step was pooled with the already transferred supernatants from the steps before. The adherent MKN45 cells were detached with 80 μl Cell Dissociation Buffer (Gibco) and transferred to the FACS plate as well. Plates were centrifuged for 4 min at 400 g. Cell pellets were washed once again with 150 μl of FACS-buffer. After centrifugation (400 g for 4 min), the supernatant was removed and cells were re-suspended by careful vortexing. The cell suspension per well was split in two and the assay plates were centrifuged again. 25 μl of the antibody mix (antibodies directed against CD4, CD8, PD-1 (Biolegend, #329920) and PD-L1 (Biolegend #329708)) were added per well. To remove unbound antibody, the cells were washed twice with 150 μl FACS buffer per well. After centrifugation (400 g for 4 min), the supernatant was removed and cells were re-suspended in 120 μl FACS buffer.

[0360] Figure 2 displays a dose-dependent up-regulation of PD-1 receptor on CD8+ (A) and on CD4+ (B) T cells as well as of PD-L1 on tumor cells that resisted to killing (C) and were harvested after 48 h of incubation.

Example 2: CEA TCB-mediated tumor lysis leads to up-regulation of PD-1 on human T cells and of PD-L1 on surviving tumor cells in vivo

[0361] Nonclinical in vivo pharmacology studies were conducted using a human colon carcinoma xenograft tumor model (LS 174T-fluc2) in immunodeficient NOG mice. Two different experimental settings were used: 1) tumor cells were co-grafted with human PBMCs (subcutaneous [SC]) at different E:T ratios, and 2) tumor cells were injected SC followed by intraperitoneal (IP) transfer of PBMCs once tumors were established (reached a palpable mass).

Xenograft Experiment with Co-Grafting of Effector Cells

[0362] Results of CEA TCB in vivo efficacy obtained upon co-grafting of LS 174T-fluc2 cells with human PBMCs at an effector:target ratio (E:T) 5:1 are displayed in Figure 3.

[0363] When administered one day after tumor/PBMCs co-grafting, both doses of CEA TCB (0.5 and 2.5 mg/kg) induced tumor regression (Figure 3A). When administered seven days after the co-grafting, only the higher dose (2.5 mg/kg) induced tumor regression, whereas the lower dose (0.5 mg/kg) was unable to induce tumor regression but led to tumor growth control and tumor stasis (Figure 3B).

[0364] The histological analysis of tumors collected at termination of the same experiment confirmed that both early (Day 1) treatment with 0.5 mg/kg CEA TCB and delayed (Day 7) treatment with 2.5 mg/kg CEA TCB led to complete elimination of CEA-expressing tumor cells and resulted in prominent T-cell infiltration into tumors (not shown). Whereas high-CEA expression on tumor cells along with variable T-cell infiltration was detected in control tumors (vehicle and untargeted MCSP TCB), CEA TCB treatment (0.5 and 2.5 mg/kg) led to a complete elimination of CEA-expressing tumor cells along with formation of necrotic/fibrotic areas filled with infiltrating T-cells (not shown). The staining of the same tumors with anti-PD-Ll antibody demonstrated a strong induction of intra-tumoral PD-L1 expression upon CEA TCB-treatment as compared to vehicle control (Figure 4).

Xenograft Experiment with IP Transfer of Effector Cells

[0365] The in vivo efficacy of CEA TCB was also evaluated in xenograft models, in which tumor cells were injected SC followed by IP transfer of PBMCs once tumors reached a palpable mass. CEA TCB (2.5 mg/kg IV bi-weekly) led to significant tumor regression (reduction of tumor weight, Figure 5) and to an increase in T-cell infiltration into tumors (flow cytometry analysis (Figure 6) and histology (not shown)), with T-cells having activated phenotype (as shown by flow cytometry analysis indicating upregulation of CD25 and CD69 activation markers, not shown). This was in contrast to the vehicle (PBS) and MCSP TCB (untargeted control), where only a low number of T-cells infiltrated tumors and showed a resting phenotype.

[0366] Additional experiments studying PD-1 and CD69 biomarkers showed that, compared to vehicle, CEA TCB induced up-regulation of the early activation marker CD69 and of PD-1 (Figure 7L, arrow) on tumor infiltrating T-cells. In contrast, peripheral blood T-cells showed a resting phenotype in both groups (Figure 7 A-F).

[0367] The staining of the same tumors with anti-PD-Ll antibody showed a strong induction of intra-tumor PD-L1 expression upon CEA TCB-treatment as compared to vehicle control.

Xenograft Experiment in Fully Humanized Mice

[0368] The antitumor activity of CEA TCB was assessed in vivo in a humanized mouse model bearing the CEA-expressing MKN45 cancer cell line subcutaneously.

[0369] CEA TCB was injected IV (2.5 mg/kg, twice/week) on Day 11 after tumor cell injection, and tumor growth was measured twice weekly by caliper. Figure 8 shows individual tumor volumes at study termination; a statistically significant ($p < 0.05$) difference in tumor volume was found.

[0370] In addition to tumor volume measurements, tumors, blood, spleen, and lymph nodes were collected at study termination (corresponding to 32 days after tumor cell injection) and analyzed by flow cytometry. CEA TCB treatment resulted in a significant increase of human T-cell frequencies in the tumor and changed the ratio of CD8+/CD4+ T-cells in favor of CD8+ T-cells (Figure 9 A and B). At the same time, both CD4+ and CD8+ T-cells exhibited an activated phenotype in the CEA TCB-treated tumors shown by up-regulation of CD69 and PD-1 expression on intratumoral CD4+ and CD8+ T-cells (Figure 9 C and D). CD8+ T-cells revealed an up-regulation of the intracellular Granzyme B (GZB) expression as well as a higher proliferation rate (Ki-67 marker). Analyses of spleen and blood samples of CEA TCB-treated animals did not show any difference in terms of T-cell frequencies, T-cell ratio, or activation status as compared to vehicle.

[0371] Thus, the immunological changes triggered by CEA TCB treatment were tumor-specific indicating that the cross-linking and activation of human T-cells occurs exclusively in CEA expressing tumors and not in other areas that are negative for CEA like blood and spleen.

[0372] Histological analysis of tumors collected at study termination confirmed the results of flow cytometry (not shown). Whereas human CEA was expressed in tumors of both vehicle and CEA TCB-treated groups, the size of tumor was significantly reduced by CEA TCB treatment. Moreover, vehicle tumors showed low infiltration of human immune cells that were mostly confined to stromal areas and the tumor border, whereas the CEA TCB-treated groups displayed a strong immune cell infiltration through the whole tumor area and not only in the periphery. In addition, human T-cells displayed increased expression of GZB within tumors. The staining of the same tumors with anti-PD-LI antibody demonstrated a strong induction of intra-tumoral PD-L1 expression upon CEA TCB treatment compared with the vehicle control (Figure 10).

Example 3: Assessment of in vivo anti-tumor activity upon combination of CEA TCB with anti-PD-LI blocking antibody in gastric carcinoma mouse model

[0373] The anti-tumor efficacy of CEA TCB in combination with anti-human PD-L1 blocking antibody was assessed in fully humanized mice bearing the gastric carcinoma tumor cell line (MKN45).

[0374] An anti-mouse PD-L1 surrogate antibody based on the YW243.55.S70 PD-L1 antibody described in WO 2010/077634 (sequence shown in Figure 11) was generated for use in vivo tumor models. This antibody contained a DAPG mutation to abolish FcγR interaction. The variable region of YW243.55.S70 was attached to a murine IgG1 constant domain with DAPG Fc mutations.

[0375] The polypeptide sequences of YW243.55.S70 PD-L1 muIgG1 are as follows:

YW243.55.S70 PD-L1 muIgG1 DAPG HC (SEQ ID NO:36):

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGG

STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQG

TLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGV

HTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCI

CTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDAPEVQFSWFVDDVEVHTAQT

QPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFGAPIEKTISKTKGRPKAPQ

VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSY

FVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

YW243.55.S70 PD-L1 muIgG1 LC (SEQ ID NO:37):

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSG

VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRADAAPTV

SIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDST

YSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

[0376] In summary, 1 x10^6 MKN45 tumor cells were injected sub-cutaneously in fully humanized NOG mice. 7 days after tumor cell injection, mice were randomized in four groups: the first group received phosphate-buffer saline (PBS, vehicle) as control, the second group received CEA TCB (at the dose of 2.5 mg/kg, administered twice a week for 7 weeks, 15 administrations in total), the third group received anti-PD-Ll antibody (at the dose of 10 mg/kg, administered once a week for 7 weeks, 8 administrations in total), and the fourth group received a combination of CEA TCB and anti-PD-Ll antibody, administered concomitantly with the respective dose and schedules used in the single therapeutic groups for 7 weeks (8 administrations of anti-PD-Ll antibody 10 mg/kg given once a week and 15 administrations of CEA TCB 2.5 mg/kg given twice a week).

[0377] The results of this study show a significant increase in anti-tumor activity upon combination of CEA TCB with anti-PD-Ll blocking antibody as compared to CEA TCB single agent (Figure 11). Treatment with anti-PD-Ll antibody as single agent did not show any anti-tumor activity. No overt test item-related toxicities were observed following treatment with the combination.

[0378] A second study was designed to assess whether, after a first line treatment with CEA TCB monotherapy, the combination of CEA TCB with a-PD-L1 could exert superior anti-tumor activity than continuing treatment with CEA TCB as monotherapy.

[0379] For this study, 1 x10^6 MKN45 tumor cells were injected sub-cutaneously in fully humanized NOG mice. 10 days after tumor cell injection, mice received either phosphate-buffer saline (PBS, vehicle) as control, or CEA TCB (at the dose of 2.5 mg/kg, administered twice a week for 4 weeks, 7 administrations in total). At study day 34, CEA TCB- treated mice were randomized into two sub-groups: one continued receiving CEA TCB administration (2.5 mg/kg, twice a week), the other received CEA TCB (2.5 mg/kg, twice a week) in combination with anti-PD-Ll antibody (10 mg/kg, once a week) for 5 weeks (11 administrations in total for CEA TCB, and 6 administrations in total for anti-PD-Ll antibody).

[0380] The results clearly show that CEA TCB + anti-PD-Ll combination therapy as second line treatment mediates significant superior anti-tumor activity than CEA TCB monotherapy (Figure 12).

Example 4: Assessment of in vivo anti-tumor activity upon combination of CEA TCB with anti-PD-Ll blocking antibody in colorectal carcinoma mouse model

[0381] The anti-tumor efficacy of CEA TCB in combination with anti- PD-L1 blocking antibody was also assessed in fully immunocompetent mice bearing the colorectal carcinoma tumor cell line MC38 engineered in-house to express human CEA on the cell surface (MC38-huCEA).

[0382] In summary, 0.5 x10^6 MC38-huCEA tumor cells were injected sub-cutaneously in fully immunocompetent C57BL/6 transgenic for human CEA (huCEA Tg mice). 17 days after tumor cell injection, mice were randomized in four groups for first line treatment: the first group received phosphate-buffer saline (PBS, vehicle) as control, the second group received CEA TCB (at the dose of 2.5 mg/kg, administered twice a week for 4 weeks, 7 administrations in total), the third group received an anti-mouse PD-L1 antibody (at the dose of 10 mg/kg, administered once a week for 4 weeks, 4 administrations in total), and the fourth group received a combination of CEA TCB and anti- PD-L1 antibody, administered concomitantly with the respective dose and schedules used in the single therapeutic groups for 4 weeks (4 administrations of anti-PD-Ll 10 mg/kg given once a week and 7 administrations of CEA TCB 2.5 mg/kg given twice a week).

[0383] The results show a significant increase in anti-tumor activity upon combination of CEA TCB with anti-PD-Ll blocking antibody as compared to CEA TCB and anti-PD-Ll single agents (Figure 13). Moreover, at study day 42, 4 out of 9 mice from the combination treated group were tumor free, while no tumor-free mice were observed upon CEA TCB treatment, and only 1 out of 8 tumor-free mice were observed upon anti-PD-Ll therapy.

[0384] No overt test item-related toxicities were observed following treatment with the combination.

[0385] At study day 42, sub-cutaneous tumors were surgically removed from all groups and mice were left untreated for 5 weeks. After this resting period, first line- treated mice plus two groups of naive huCEA Tg mice (one left untouched, and the other previously submitted to a surgical cut on the flank to mimic surgical intervention) were injected sub-cutaneously with 0.5 x10^6 MC38-huCEA tumor cell lines on the opposite flank. No therapy injection was applied to any of the groups, while tumor growth was monitored. The results show that in all first line treated mice, tumor protection upon tumor rechallenge was observed, with significant tumor control as compared to naive mice. The group who had

received the combination of CEA TCB with anti-PD-Ll as first line treatment, showed 3 out of 8 tumor-free mice at study day 37 upon tumor rechallenge. These data indicate that in the MC38-huCEA model, the combination of CEA TCB with anti-PD-Ll antibody not only improved first line treatment response, but also improved anti-tumor protection upon subsequent tumor challenge.

Example 5: An open-label, multi-center, dose escalation and expansion Phase IB study to evaluate the safety, pharmacokinetics and therapeutic activity of CEA TCB in combination with atezolizumab in patients with locally advanced and/or metastatic CEA-positive solid tumors

[0386] Data discussed above demonstrate that CEA TCB and atezolizumab act synergistically in their anti-cancer properties and collectively their combination could provide meaningful clinical benefit in patients with cancer.

[0387] An open-label, multi-center, dose escalation and expansion Phase Ib clinical study of CEA TCB in combination with atezolizumab is conducted. Each treatment cycle is 21 days in duration and consists of IV infusions of CEA TCB given weekly (QW) ($\pm$ 1 day) in combination with atezolizumab given every 3 weeks (Q3W) ($\pm$ 2 days). Patients receive atezolizumab (1200 mg fixed dose) IV on Day 1 of each cycle, followed by CEA TCB at least one hour later, given IV on Day 1, Day 8 and Day 15 of each cycle.

[0388] For CEA TCB, the starting dose is 5 mg administered on a QW schedule, to be administered at least one hour after the administration of 1200 mg of atezolizumab when they are both administered on the same day (Day 1 of each cycle).

[0389] Patients are treated until loss of clinical benefit, unacceptable toxicities, or withdrawal of consent. In case one of the treatments is permanently discontinued, treatment with the other drug alone may be continued.

[0390] Key eligibility criteria include confirmed locally advanced and/or metastatic solid tumor in patients who have progressed on a standard therapy, are intolerant to standard therapy, and/or are non-amenable to standard therapy; Eastern Cooperative Oncology Group

[0391] (ECOG) Performance Status (PS) 0-1; and locally confirmed CEA expression in tumor tissue ($\geq$ 50% of tumor cells staining with at least moderate to high intensity of CEA expression for all solid tumors with exception of NSCLC $\geq$ 20% of tumor cells staining with at least moderate to high intensity of CEA expression) or centrally confirmed CEA expression.

[0392] Primary objectives of this study are safety and tolerability, with secondary objectives of PK and clinical activity (objective overall response rate (ORR), disease control rate (DCR; defined as response rate [RR] + stable disease rate [SDR]), progression-free survival (PFS) and overall survival (OS) data according to Response Evaluation Criteria in Solid Tumors (RECIST), Version 1.1 criteria and modified RECIST criteria).

[0393] Tumor response is evaluated according to RECIST v1.1 and modified RECIST criteria using unidimensional measurement such as computed tomography (CT) scan or magnetic resonance imaging.

Results

[0394] As of 15 November 2016, 30 patients have been treated at the following dose cohort levels:

5 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 4 patients
10 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 4 patients
20 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 4 patients
40 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 6 patients
80 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 5 patients
160 mg of CEA TCB QW+ 1200 atezolizumab Q3W: 7 patients

[0395] The combination treatment was at current dose levels (up to 160 mg CEA TCB QW + 1200 mg atezolizumab Q3W) safe and well tolerated. No patients were discontinued due to a treatment related safety event.

[0396] All patients in this study had an FDG PET scan tumor assessment at week 4 and CT scan tumor assessment at week 8 and then every 8 weeks.

[0397] At 20 mg CEA TCB, 2/4 patients have experienced prolonged stable disease according to RECIST criteria. One patient with Pancreas adenocarcinoma has been in the study for 32 weeks, the patient experienced stable metabolic response assessed by FDG PET at week 4. The second patient, a gastro-esophageal junction adenocarcinoma with MSI instability has been in the study for 30 weeks. This patient experienced a partial metabolic response at week 4 FDG PET assessment.

[0398] At 40 mg CEA TCB, 1/6 patients experienced prolonged stable disease according to RECIST criteria and it's been on study for 20 weeks. This patient, a colorectal carcinoma patient, was previously treated in the monotherapy trial (BP29541) and received 31 cycles of treatment.

[0399] At 80 mg CEA TCB, 5/5 patients experienced tumor volume reduction at week 8 CT scan and metabolic partial response at week 4 FDG PET scan. In this cohort 4/5 patients were colorectal carcinoma patients and 1 patient with ampulla Vater carcinoma.

[0400] In summary, the clinical data generated so far in this trial showed promising relevant clinical antitumor activity in 2 /4 patients treated at 20 mg CEA TCB + 1200 mg atezolizumab, 1/6 patients treated at 40 mg CEA TCB + 1200 mg atezolizumab, and 5/5 patients treated at 80 mg CEA TCB + 1200 mg atezolizumab.

[0401] The data discussed above regarding the 80 mg CEA TCB cohort in a population that do not respond to anti PD-1 or anti PD-L1 treatment, supports that CEA TCB and atezolizumab act synergistically in their anti-cancer properties and collectively their combination could provide meaningful clinical benefit in patients with cancer.

[0402] The following numbered paragraphs describe additional aspects and embodiments of the invention:

1. A method for treating or delaying progression of cancer in an individual comprising administering to the individual an effective amount of a human PD-1 axis binding antagonist and an anti-CEA/anti-CD3 antibody.

2. The method of paragraph 1, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

3. The method of paragraph 1 or 2, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

4. The method of paragraph 3, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to its ligand binding partners.

5. The method of paragraph 3 or 4, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

6. The method of any one of paragraphs 3-5, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

7. The method of any one of paragraphs 3-6, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

8. The method of any one of paragraphs 3-7, wherein the PD-1 binding antagonist is an antibody.

9. The method of any one paragraphs 3-8, wherein the PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

10. The method of paragraph 2, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

11. The method of paragraph 10, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

12. The method of paragraph 10 or 11, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

13. The method of any one of paragraphs 10-12, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

14. The method of any one of paragraphs 10-13, wherein the PD-L1 binding antagonist is an antibody.

15. The method of any one of paragraphs 10-14, wherein the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

16. The method of paragraph 14, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24.

17. The method of paragraph 14 or 16, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 or 26 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

18. The method of paragraph 2, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

19. The method of paragraph 18, wherein the PD-L2 binding antagonist is an antibody.

20. The method of paragraph 18, wherein the PD-L2 binding antagonist is an immunoadhesin.

21. The method of any one of paragraphs 1-19, wherein the PD-1 axis binding antagonist is an antibody and comprises an aglycosylation site mutation.

22. The method of paragraph 21, wherein the aglycosylation site mutation is a substitution mutation.

23. The method of paragraph 22, wherein the substitution mutation is at amino acid residue N297, L234, L235, and/or D265 (EU numbering).

24. The method of paragraph 21 or 22, wherein the substitution mutation is selected from the group consisting of N297G, N297A, L234A, L235A, and D265A.

25. The method of any one of paragraphs 21-23, wherein the substitution mutation is a D265A mutation and an N297G mutation.

26. The method of any one of paragraphs 1-25, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, and a second antigen binding domain that binds to CEA.

27. The method of paragraph 26, wherein the first antigen binding domain comprises a heavy chain variable region

(V$_H$CD3) and a light chain variable region (V$_L$CD3), and the second antigen binding domain comprises a heavy chain variable region (V$_H$CEA) and a light chain variable region (V$_L$CEA).

28. The method of paragraph 26 or 27, wherein the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) comprising HVR-H1 sequence of SEQ ID NO:44, HVR-H2 sequence of SEQ ID NO:45, and HVR-H3 sequence of SEQ ID NO:46; and/or a light chain variable region (V$_L$CD3) comprising HVR-L1 sequence of SEQ ID NO:47, HVR-L2 sequence of SEQ ID NO:48, and HVR-L3 sequence of SEQ ID NO:49.

29. The method of any one of paragraphs 26-28, wherein the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) comprising the amino acid sequence of SEQ ID NO:50 and/or a light chain variable region (V$_L$CD3) comprising the amino acid sequence of SEQ ID NO:51.

30. The method of any one of paragraphs 26-29, wherein the second antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V$_L$CEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

31. The method of any one of paragraphs 26-30, wherein the second antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V$_L$CEA) comprising the amino acid sequence of SEQ ID NO:35.

32. The method of any one of paragraphs 26-31, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a third antigen binding domain that binds to CEA.

33. The method of paragraph 32, wherein the third antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V$_L$CEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43.

34. The method of paragraph 32 or 33, wherein the third antigen binding domain comprises a heavy chain variable region (V$_H$CEA) comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V$_L$CEA) comprising the amino acid sequence of SEQ ID NO:35.

35. The method of any one of paragraphs 26-34, wherein the antigen binding domains are Fab molecules.

36. The method of any one of paragraphs 26-35, wherein the first antigen binding domain is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third, if present, antigen binding domain is a conventional Fab molecule.

37. The method of paragraphs 35 or 36, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain.

38. The method of any one of paragraphs 26-37, wherein the anti-CEA/anti-CD3 bispecific antibody further comprises an Fc domain composed of a first and a second subunit capable of stable association.

39. The method of paragraph 38, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, and the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, and the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain.

40. The method of paragraphs 38 or 39, wherein (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

41. The method of any one of paragraphs 38-40, wherein the Fc domain is an IgG, specifically an IgG$_1$ or IgG$_4$, Fc domain.

42. The method of any one of paragraphs 38-41, wherein the Fc domain is a human Fc domain.

43. The method of any one of paragraphs 38-42, wherein the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain.

44. The method of paragraph 43, wherein in the CH3 domain of the first subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second

subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

45. The method of any one of paragraphs 38-44, wherein the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG_1 Fc domain.

46. The method of any one of paragraphs 38-45, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

47. The method of paragraph 46, wherein said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (EU numbering).

48. The method of any one of paragraphs 38-47, wherein each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (EU numbering).

49. The method of any one of paragraphs 1-48, wherein the cancer is a CEA-positive cancer.

50. The method of any one of paragraphs 1-49, wherein the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer.

51. The method of any one of paragraphs 1-50, wherein the individual has cancer or has been diagnosed with cancer.

52. The method of any one of paragraphs 1-51, wherein cancer cells in the individual express PD-L1.

53. The method of any one of paragraphs 1-52, wherein the human PD-1 axis binding antagonist is administered at a dose of about 1200 mg every three weeks.

54. The method of any one of paragraphs 1-52, wherein the anti-CEA/anti-CD3 bispecific antibody is administered at a dose of about 5 mg to about 100 mg every week.

55. The method of any one of paragraphs 1-53, comprising treatment cycles wherein the individual is administered, on day 1 of each cycle, a human PD-1 axis binding antagonist at a dose of about 1200 mg, and on days 1, 8, and 15 of each cycle, an anti-CEA/anti-CD3 antibody at a dose of about 5 mg, about 10 mg, about 20 mg, about 40 mg, about 80 mg or about 160 mg, particularly at least about 80 mg, each cycle being repeated every 21 days.

56. The method of any one of paragraphs 1-55, wherein the individual is refractory to a chemotherapeutic agent treatment.

57. The method of any one of paragraphs 1-55, wherein the individual is intolerant to a chemotherapeutic agent treatment.

58. The method of any one of paragraphs 1-57, wherein the treatment results in a response in the individual.

59. The method of any one of paragraphs 1-58, wherein the PD-1 axis binding antagonist and/or the anti-CEA/anti-CD3 bispecific antibody are administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally.

60. The method of any one of paragraphs 1-59, further comprising administering a chemotherapeutic agent for treating or delaying progression of cancer.

61. A method of enhancing immune function in an individual having cancer comprising administering an effective amount of a PD-1 axis binding antagonist and an anti-CEA/anti-CD3 bispecific antibody.

62. The method of paragraph 61, wherein CD8 T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity relative to prior to the administration of the PD-1 axis binding antagonist and the anti-HER2 antibody.

63. The method of paragraph 61 or 62, wherein the number of CD8 T cells is elevated relative to prior to administration of the combination.

64. The method of paragraph 62 or 63, wherein the CD8 T cell is an antigen-specific CD8 T cell.

65. The method of any one of paragraphs 61-64, wherein Treg function is suppressed relative to prior to the administration of the combination.

66. The method of any one of paragraphs 61-65, wherein T cell exhaustion is decreased relative to prior to the administration of the combination.

67. Use of a human PD-1 axis binding antagonist in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier.

68. Use of an anti-CEA/anti-CD3 bispecific antibody in the manufacture of a medicament for treating or delaying progression of cancer in an individual, wherein the medicament comprises the anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier, and wherein the treatment comprises administration of the medicament in combination with a composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

69. A composition comprising a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable

carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier.

70. A composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier for use in treating or delaying progression of cancer in an individual, wherein the treatment comprises administration of said composition in combination with a second composition, wherein the second composition comprises a human PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier.

71. A kit comprising a medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

72. A kit comprising a first medicament comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier, and a second medicament comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier.

73. The kit of paragraph 72, wherein the kit further comprises a package insert comprising instructions for administration of the first medicament and the second medicament for treating or delaying progression of cancer in an individual.

74. A kit comprising a medicament comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5               10              15


Ser Leu Arg Leu Asp Cys Lys Ala Ser Gly Ile Thr Phe Ser Asn Ser
        20              25              30


Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35              40              45


Ala Val Ile Trp Tyr Asp Gly Ser Lys Arg Tyr Tyr Ala Asp Ser Val
        50              55              60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Phe
65              70              75              80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85              90              95


Ala Thr Asn Asp Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
        100             105             110


Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
        115             120             125


Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
        130             135             140


Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
```

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Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
165 170 175

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys
180 185 190

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
195 200 205

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala
210 215 220

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
225 230 235 240

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
245 250 255

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
260 265 270

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
275 280 285

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
290 295 300

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
305 310 315 320

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
325 330 335

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
340 345 350

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
355 360 365

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
370 375 380

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
385 390 395 400

```
         Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
                     405             410             415

         Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                     420             425             430

         Ser Leu Ser Leu Ser Leu Gly Lys
                     435             440


         <210>  2
         <211>  214
         <212>  PRT
         <213>  Artificial Sequence

         <220>
         <223>  Synthetic Construct

         <400>  2

         Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
         1               5               10              15

         Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
                     20              25              30

         Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
                     35              40              45

         Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
                 50              55              60

         Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
         65              70              75              80

         Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Ser Asn Trp Pro Arg
                     85              90              95

         Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
                     100             105             110

         Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
                     115             120             125

         Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
                 130             135             140

         Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
         145             150             155             160

         Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
```

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                                165                      170                      175


         Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
                 180                 185                 190


         Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
                 195                 200                 205


         Phe Asn Arg Gly Glu Cys
             210


         <210>  3
         <211>  118
         <212>  PRT
         <213>  Artificial Sequence

         <220>
         <223>  Synthetic Construct

         <400>  3

         Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
         1               5                   10                  15


         Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
                 20                  25                  30


         Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
                 35                  40                  45


         Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
                 50                  55                  60


         Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
         65                  70                  75                  80


         Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95


         Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
                 100                 105                 110


         Leu Val Thr Val Ser Ala
                 115


         <210>  4
         <211>  108
         <212>  PRT
         <213>  Artificial Sequence

         <220>
```

<223>   Synthetic Construct

<400>   4

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5               10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30


Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

<210>   5
<211>   10
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<220>
<221>   VARIANT
<222>   (6)..(6)
<223>   Asp or Gly

<400>   5

```
Gly Phe Thr Phe Ser Xaa Ser Trp Ile His
1               5               10
```

<210>   6
<211>   18
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<220>
<221>   VARIANT

```
<222>  (4)..(4)
<223>  Ser or Leu

<220>
<221>  VARIANT
<222>  (10)..(10)
<223>  Thr or Ser

<400>  6

Ala Trp Ile Xaa Pro Tyr Gly Gly Ser Xaa Tyr Tyr Ala Asp Ser Val
1               5                   10              15


Lys Gly



<210>  7
<211>  9
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  7

Arg His Trp Pro Gly Gly Phe Asp Tyr
1               5



<210>  8
<211>  25
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  8

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10              15


Ser Leu Arg Leu Ser Cys Ala Ala Ser
            20                  25


<210>  9
<211>  13
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  9

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
1               5                   10
```

```
<210>   10
<211>   32
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   10

Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln
1               5                   10                  15


Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
                20                  25                  30


<210>   11
<211>   11
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   11

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
1               5                   10


<210>   12
<211>   11
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct


<220>
<221>   VARIANT
<222>   (5)..(5)
<223>   Asp or Val

<220>
<221>   VARIANT
<222>   (6)..(6)
<223>   Val or Ile

<220>
<221>   VARIANT
<222>   (7)..(7)
<223>   Ser or Asn

<220>
<221>   VARIANT
<222>   (9)..(9)
<223>   Ala or Phe

<220>
```

```
<221>  VARIANT
<222>  (10)..(10)
<223>  Val or Leu

<400>  12

Arg Ala Ser Gln Xaa Xaa Xaa Thr Xaa Xaa Ala
1               5                   10


<210>  13
<211>  7
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct


<220>
<221>  VARIANT
<222>  (4)..(4)
<223>  Phe or Thr

<220>
<221>  VARIANT
<222>  (6)..(6)
<223>  Tyr or Ala

<400>  13

Ser Ala Ser Xaa Leu Xaa Ser
1               5


<210>  14
<211>  9
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct


<220>
<221>  VARIANT
<222>  (3)..(3)
<223>  Tyr, Gly, Phe or Ser

<220>
<221>  VARIANT
<222>  (4)..(4)
<223>  Leu, Tyr, Phe or Trp

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<222>  (5)..(5)
<223>  Tyr, Asn, Ala, Thr, Gly, Phe or Ile

<220>
<221>  VARIANT
<222>  (6)..(6)
<223>  His, Val, Pro, Thr or Ile
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<220>
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<222> (8)..(8)
<223> Ala, Trp, Arg, Pro or Thr

<400> 14

Gln Gln Xaa Xaa Xaa Xaa Pro Xaa Thr
1   5

<210> 15
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 15

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys
            20

<210> 16
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 16

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1   5   10   15

<210> 17
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 17

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1   5   10   15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
            20   25   30

<210> 18

<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 18

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1   5   10

<210> 19
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 19

Gly Phe Thr Phe Ser Asp Ser Trp Ile His
1   5   10

<210> 20
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 20

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
1   5   10   15

Lys Gly

<210> 21
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 21

Arg His Trp Pro Gly Gly Phe Asp Tyr
1   5

<210> 22
<211> 11
<212> PRT
<213> Artificial Sequence

```
<220>
<223>  Synthetic Construct

<400>  22

Arg Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1               5                   10


<210>  23
<211>  7
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  23

Ser Ala Ser Phe Leu Tyr Ser
1               5


<210>  24
<211>  9
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  24

Gln Gln Tyr Leu Tyr His Pro Ala Thr
1               5


<210>  25
<211>  118
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  25

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30


Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45


Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
            50                  55                  60
```

```
Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser
        115


<210>  26
<211>  122
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  26

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30


Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45


Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
        50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser Ala Ser Thr Lys
        115                 120


<210>  27
<211>  11
<212>  PRT
<213>  Artificial Sequence
```

```
<220>
<223>   Synthetic Construct

<400>   27

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1               5                   10


<210>   28
<211>   10
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   28

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1               5                   10


<210>   29
<211>   30
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   29

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
            20                  25                  30


<210>   30
<211>   14
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
1               5                   10


<210>   31
<211>   15
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct
```

<400> 31

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
1   5   10   15

<210> 32
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20   25   30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35   40   45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
        50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100   105   110

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
            115   120   125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
        130   135   140

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145   150   155   160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165   170   175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180   185   190

```
      Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
              195                 200             205

      Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
              210                 215             220

      His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
      225                 230             235                 240

      Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                      245             250                 255

      Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                  260             265             270

      Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
              275                 280             285

      Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val
              290                 295             300

      Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
      305                 310                 315                 320

      Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                      325             330                 335

      Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
              340                 345                 350

      Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
              355                 360                 365

      Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
              370                 375                 380

      Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
      385                 390                 395                 400

      Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                      405                 410                 415

      Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                      420                 425                 430

      Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
```

<210> 33
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 33

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
            35   40   45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
        50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100   105   110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
            115   120   125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
        130   135   140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145   150   155   160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165   170   175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180   185   190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
            195   200   205

Phe Asn Arg Gly Glu Cys
    210

<210>   34
<211>   121
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   34

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1   5   10   15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
            20   25   30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35   40   45

Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
    50   55   60

Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65   70   75   80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
            85   90   95

Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
            100   105   110

Gln Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210>   35
<211>   108
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   35

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr

| | | 20 | | | | | 25 | | | | | 30 | | |

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
        50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
            85   90   95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100   105

<210> 36
<211> 442
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 36

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20   25   30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
        50   55   60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
            85   90   95

Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
            100   105   110

Leu Val Thr Val Ser Ala Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro
            115   120   125

```
Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly
    130             135             140

Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn
145             150             155             160

Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165             170             175

Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr
            180             185             190

Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser
        195             200             205

Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro
    210             215             220

Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro
225             230             235             240

Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys
            245             250             255

Val Val Val Asp Ile Ser Lys Asp Ala Pro Glu Val Gln Phe Ser Trp
            260             265             270

Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu
            275             280             285

Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met
    290             295             300

His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser
305             310             315             320

Ala Ala Phe Gly Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
            325             330             335

Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln
            340             345             350

Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe
            355             360             365

Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu
```

<pre>
         370   375   380

     Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe
     385   390   395   400

     Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn
                     405   410   415

     Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr
                 420   425   430

     Glu Lys Ser Leu Ser His Ser Pro Gly Lys
             435   440
</pre>

<210> 37
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 37

<pre>
     Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
     1   5   10   15

     Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Ser Thr Ala
                 20   25   30

     Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
                 35   40   45

     Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
         50   55   60

     Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
     65   70   75   80

     Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Leu Tyr His Pro Ala
                 85   90   95

     Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Ala Asp Ala Ala
                 100   105   110

     Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
                 115   120   125

     Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
         130   135   140
</pre>

88

```
Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145                 150                 155                 160


Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
                165                 170                 175


Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
            180                 185                 190


Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
        195                 200                 205


Phe Asn Arg Asn Glu Cys
    210
```

```
<210>   38
<211>   5
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   38

Glu Phe Gly Met Asn
1               5


<210>   39
<211>   17
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   39

Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe Lys
1               5                   10                  15

Gly


<210>   40
<211>   12
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   40
```

```
Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr
1               5                   10
```

```
<210>  41
<211>  11
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  41
```

```
Lys Ala Ser Ala Ala Val Gly Thr Tyr Val Ala
1               5                   10
```

```
<210>  42
<211>  7
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  42
```

```
Ser Ala Ser Tyr Arg Lys Arg
1               5
```

```
<210>  43
<211>  10
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  43
```

```
His Gln Tyr Tyr Thr Tyr Pro Leu Phe Thr
1               5                   10
```

```
<210>  44
<211>  5
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  44
```

```
Thr Tyr Ala Met Asn
1               5
```

```
<210>  45
<211>  19
```

```
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  45

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser
1               5                   10                  15


Val Lys Gly



<210>  46
<211>  14
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  46

His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr
1               5                   10


<210>  47
<211>  14
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  47

Gly Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn
1               5                   10


<210>  48
<211>  7
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  48

Gly Thr Asn Lys Arg Ala Pro
1               5


<210>  49
<211>  9
<212>  PRT
<213>  Artificial Sequence
```

<220>
<223>   Synthetic Construct

<400>   49

Ala Leu Trp Tyr Ser Asn Leu Trp Val
1   5

<210>   50
<211>   125
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   50

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr
        20   25   30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ser Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp
        50   55   60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65   70   75   80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
                85   90   95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe
        100   105   110

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120   125

<210>   51
<211>   109
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   51

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1   5   10   15

```
Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
            35                  40                  45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
            50                  55                  60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
                100                 105
```

```
<210>   52
<211>   214
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   52
```

```
Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly
1               5                   10                  15

Thr Val Thr Leu Thr Cys Gly Ser Ser Thr Gly Ala Val Thr Thr Ser
            20                  25                  30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Gly Gln Ala Phe Arg Gly
            35                  40                  45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Gly Thr Pro Ala Arg Phe
            50                  55                  60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Ala
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

Leu Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser Ser Ala
                100                 105                 110
```

```
        Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
                115                 120                 125

        Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
                130                 135                 140

        Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
        145                 150                 155                 160

        Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
                        165                 170                 175

        Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
                    180                 185                 190

        Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys
                195                 200                 205

        Val Glu Pro Lys Ser Cys
                210


<210>   53
<211>   694
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic Construct

<400>   53

        Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
        1                   5                   10                  15

        Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
                    20                  25                  30

        Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
                35                  40                  45

        Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
            50                  55                  60

        Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
        65                  70                  75                  80

        Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                        85                  90                  95
```

```
        Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
                    100             105             110

        Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
                    115             120             125

        Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
                    130             135             140

        Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
        145             150             155             160

        Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
                    165             170             175

        Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
                    180             185             190

        Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
                    195             200             205

        Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
            210             215             220

        Asp Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Leu
        225             230             235             240

        Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser
                    245             250             255

        Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr Ala Met Asn Trp Val
                    260             265             270

        Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Arg Ser
                    275             280             285

        Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg
                    290             295             300

        Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met
        305             310             315             320

        Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His
                    325             330             335

        Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr Trp Gly Gln
                    340             345             350
```

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Val Ala Ala Pro Ser Val
        355   360   365

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
        370   375   380

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
385   390   395   400

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
                405   410   415

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
        420   425   430

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
        435   440   445

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
        450   455   460

Gly Glu Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
465   470   475   480

Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
                485   490   495

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
                500   505   510

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
        515   520   525

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
        530   535   540

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
545   550   555   560

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly
                565   570   575

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        580   585   590

Pro Gln Val Tyr Thr Leu Pro Pro Cys Arg Asp Glu Leu Thr Lys Asn
        595   600   605

96

```
Gln Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
    610                 615                 620
```

```
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
625                 630                 635                 640
```

```
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
                645                 650                 655
```

```
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                660                 665                 670
```

```
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
        675                 680                 685
```

```
Ser Leu Ser Pro Gly Lys
    690
```

```
<210>  54
<211>  451
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  54
```

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15
```

```
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Glu Phe
            20                  25                  30
```

```
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45
```

```
Gly Trp Ile Asn Thr Lys Thr Gly Glu Ala Thr Tyr Val Glu Glu Phe
    50                  55                  60
```

```
Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80
```

```
Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
Ala Arg Trp Asp Phe Ala Tyr Tyr Val Glu Ala Met Asp Tyr Trp Gly
            100                 105                 110
```

Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
      115   120   125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
      130   135   140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145   150   155   160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
          165   170   175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
          180   185   190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
      195   200   205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
      210   215   220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly
225   230   235   240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
          245   250   255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
          260   265   270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
          275   280   285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
      290   295   300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305   310   315   320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Gly Ala Pro Ile
          325   330   335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
          340   345   350

Cys Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
          355   360   365

Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    370   375   380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385   390   395   400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Val Ser Lys Leu Thr Val
            405   410   415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
            420   425   430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
        435   440   445

Pro Gly Lys
    450

<210> 55
<211> 215
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 55

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Ala Ala Val Gly Thr Tyr
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
            35   40   45

Tyr Ser Ala Ser Tyr Arg Lys Arg Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Tyr Tyr Thr Tyr Pro Leu
            85   90   95

Phe Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
            100   105   110

99

```
Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
        130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

```
<210>  56
<211>  448
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  56
```

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Ser
            20                  25                  30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45

Ala Trp Ile Ser Pro Tyr Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
        50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
```

```
           Ala Arg Arg His Trp Pro Gly Gly Phe Asp Tyr Trp Gly Gln Gly Thr
                       100             105             110

   5       Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
                       115             120             125

   10      Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
                       130             135             140

           Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
           145             150             155             160

   15      Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                       165             170             175

   20      Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                       180             185             190

           Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
                       195             200             205

   25      Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
                       210             215             220

   30      His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
           225             230             235             240

   35      Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                       245             250             255

           Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
                       260             265             270

   40      Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
                       275             280             285

   45      Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val Val
                       290             295             300

   50      Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
           305             310             315             320

           Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                       325             330             335

   55      Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
                       340             345             350
```

```
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360             365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370             375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390             395                 400

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                405             410                 415

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            420             425                 430

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            435                 440                 445
```

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<210>  57
<211>  225
<212>  PRT
<213>  Homo sapiens

<400>  57

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1               5                   10                  15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            20                  25                  30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
            35                  40                  45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
        50                  55                  60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65                  70                  75                  80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                85                  90                  95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            100                 105                 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
            115                 120                 125
```

```
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    130             135             140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
    145             150             155             160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                165             170             175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            180             185             190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        195             200             205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210             215             220

Pro
225
```

Claims

1. A kit comprising a medicament comprising an anti-CEA/anti-CD3 bispecific antibody and an optional pharmaceutically acceptable carrier, and a package insert comprising instructions for administration of the medicament in combination with a composition comprising a PD-1 axis binding antagonist and an optional pharmaceutically acceptable carrier for treating or delaying progression of cancer in an individual.

2. The kit of claim 1, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist or a PD-L1 binding antagonist, optionally wherein the PD-1 binding antagonist or PD-L1 binding antagonist is an antibody.

3. The kit of claim 2, wherein the (i) PD-1 binding antagonist is selected from the group consisting of MDX 1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108, or (ii) the PD-L1 binding antagonist is selected from the group consisting of: MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

4. The kit of claim 2, wherein the antibody comprises a heavy chain comprising HVR-H1 sequence of SEQ ID NO:19, HVR-H2 sequence of SEQ ID NO:20, and HVR-H3 sequence of SEQ ID NO:21; and a light chain comprising HVR-L1 sequence of SEQ ID NO:22, HVR-L2 sequence of SEQ ID NO:23, and HVR-L3 sequence of SEQ ID NO:24, and optionally comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:25 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4.

5. The kit of claim 4, wherein the antibody comprises an aglycosylation site mutation, particularly a substitution mutation.

6. The kit of any one of claims 1-5, wherein the anti-CEA/anti-CD3 bispecific antibody comprises a first antigen binding domain that binds to CD3, a second antigen binding domain that binds to CEA, and optionally a third antigen binding domain that binds to CEA, wherein the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) and a light chain variable region (V$_L$CD3), and the second and third antigen binding domain comprises a heavy chain variable region (V$_H$CEA) and a light chain variable region (V$_L$CEA).

7. The kit of claim 6, wherein the first antigen binding domain comprises a heavy chain variable region (V$_H$CD3) comprising HVR-H1 sequence of SEQ ID NO:44, HVR-H2 sequence of SEQ ID NO:45, and HVR-H3 sequence of

SEQ ID NO:46; and/or a light chain variable region (V_LCD3) comprising HVR-L1 sequence of SEQ ID NO:47, HVR-L2 sequence of SEQ ID NO:48, and HVR-L3 sequence of SEQ ID NO:49, and optionally the first antigen binding domain comprises a heavy chain variable region (V_HCD3) comprising the amino acid sequence of SEQ ID NO:50 and/or a light chain variable region (V_LCD3) comprising the amino acid sequence of SEQ ID NO:51.

8. The kit of claim 6 or 7, wherein the second and third antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising HVR-H1 sequence of SEQ ID NO:38, HVR-H2 sequence of SEQ ID NO:39, and HVR-H3 sequence of SEQ ID NO:40; and/or a light chain variable region (V_LCEA) comprising HVR-L1 sequence of SEQ ID NO:41, HVR-L2 sequence of SEQ ID NO:42, and HVR-L3 sequence of SEQ ID NO:43, and optionally the second and third antigen binding domain comprises a heavy chain variable region (V_HCEA) comprising the amino acid sequence of SEQ ID NO:34 and/or a light chain variable region (V_LCEA) comprising the amino acid sequence of SEQ ID NO:35.

9. The kit of any one of claims 6-8, wherein the antigen binding domains are Fab molecules, optionally the first antigen binding domain is a crossover Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged, and the second and third antigen binding domain is a conventional Fab molecule.

10. The kit of any one of claims 6-9, wherein the anti-CEA/anti-CD3 bispecific antibody further comprises an Fc domain composed of a first and a second subunit capable of stable association, an optionally (i) the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding domain, the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain, or (ii) the first antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding domain, the second antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and the third antigen binding domain is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain.

11. The kit of claim 10, wherein the Fc domain is an IgG, specifically an IgG_1 Fc domain, most specifically a human IgG_1 Fc domain.

12. The kit of claim 10 or 11, wherein the Fc domain comprises a modification promoting the association of the first and the second subunit of the Fc domain, and/or wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

13. The kit of any one of claims 1-12, wherein the cancer is a CEA-positive cancer, and optionally wherein the cancer is colon cancer, lung cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, breast cancer, kidney cancer, esophageal cancer, or prostate cancer.

14. The kit of any one of claims 1-13, further comprising a medicament comprising a PD-1 axis binding antagonist, particularly a PD-1 axis binding antagonist as defined in claims 2-5, and an optional pharmaceutically acceptable carrier.
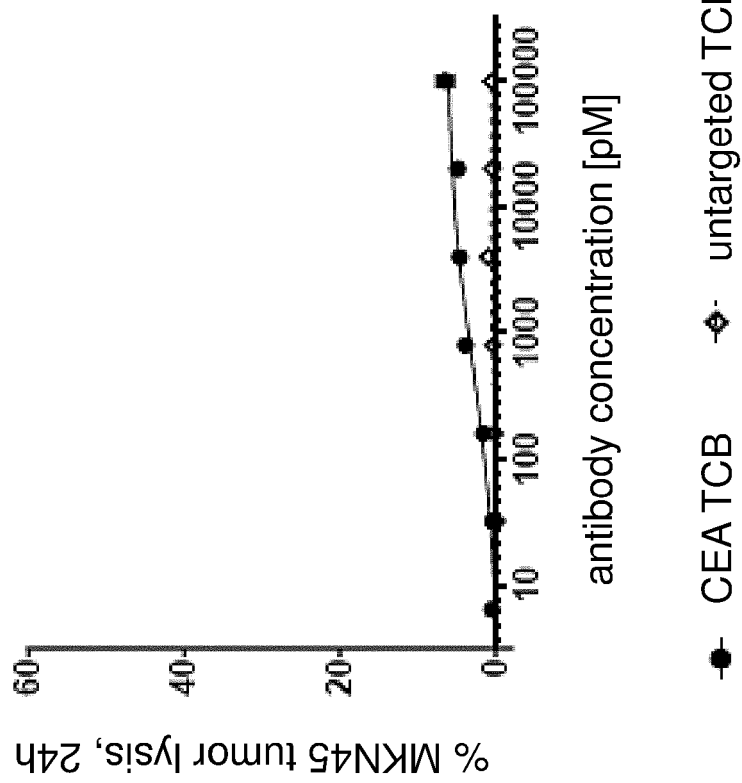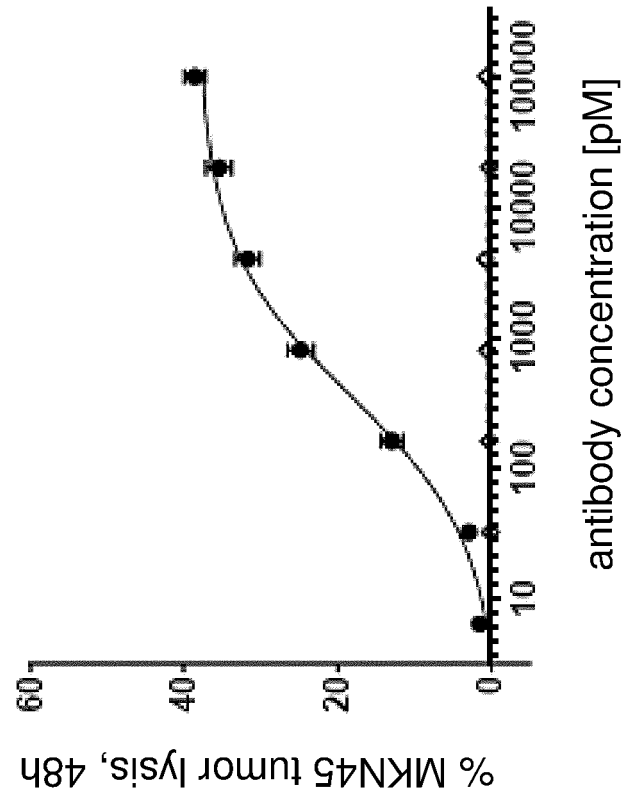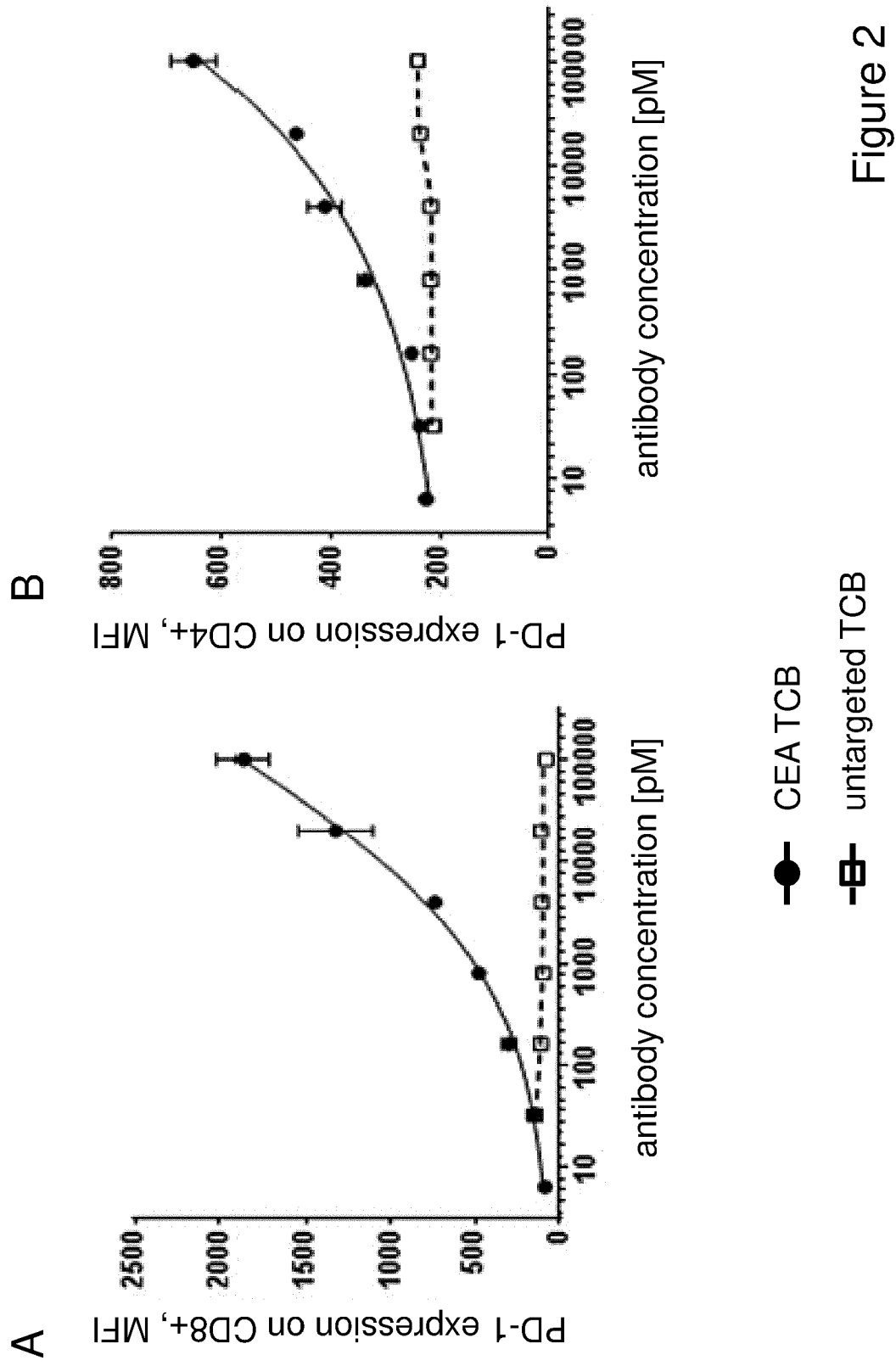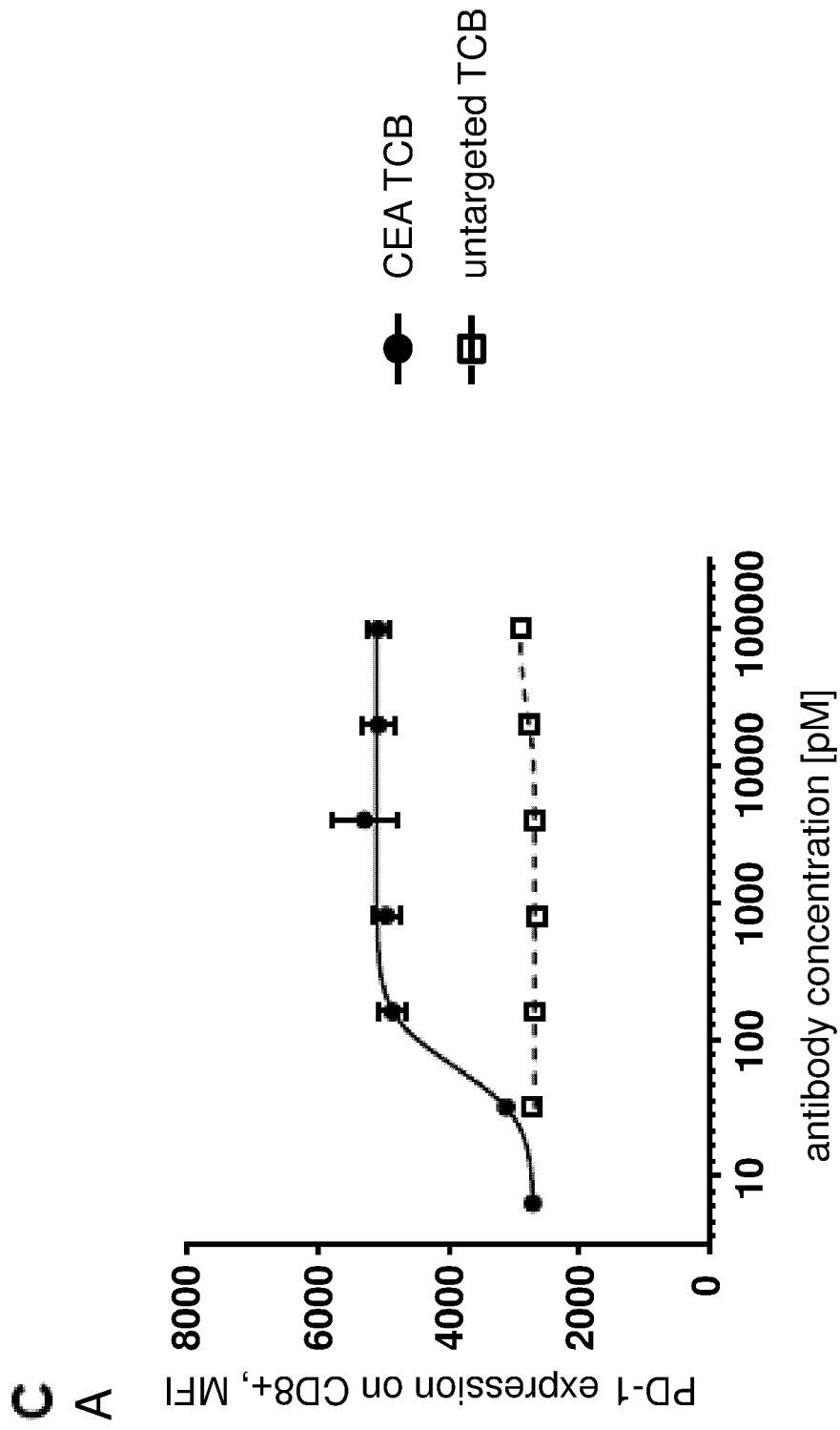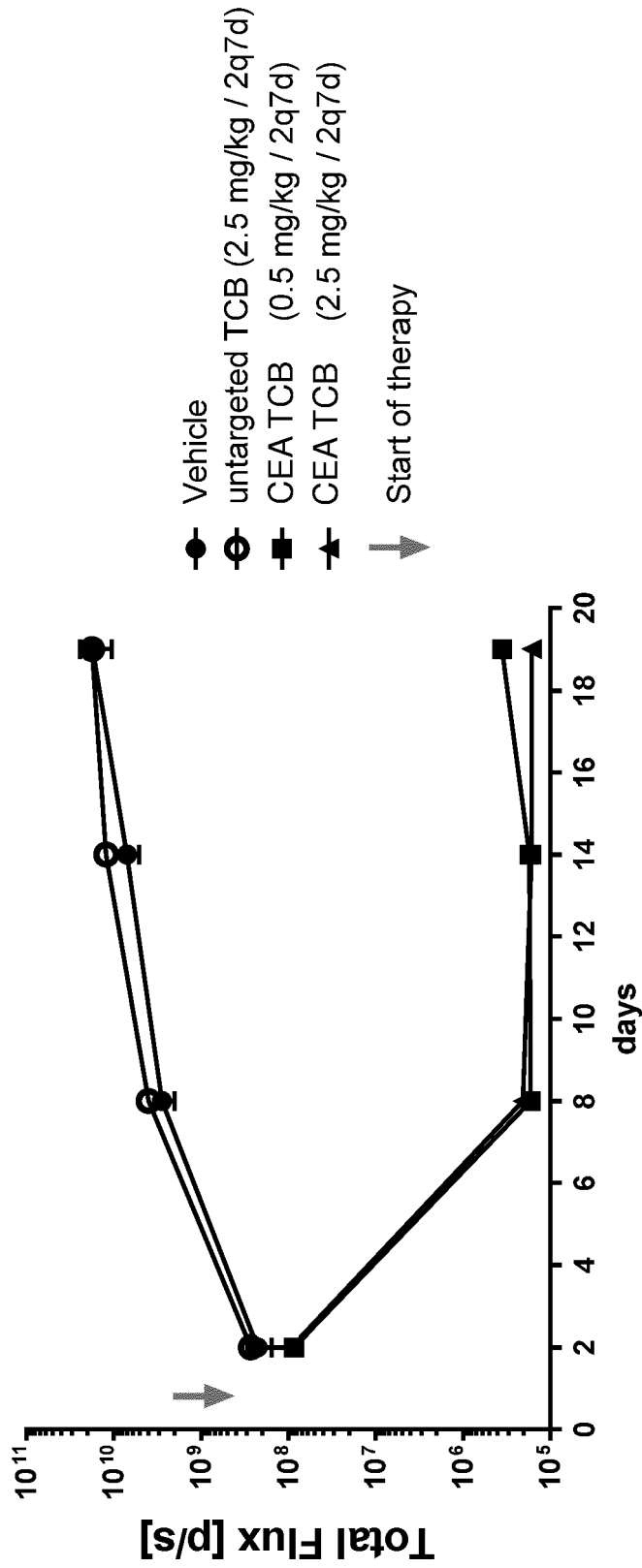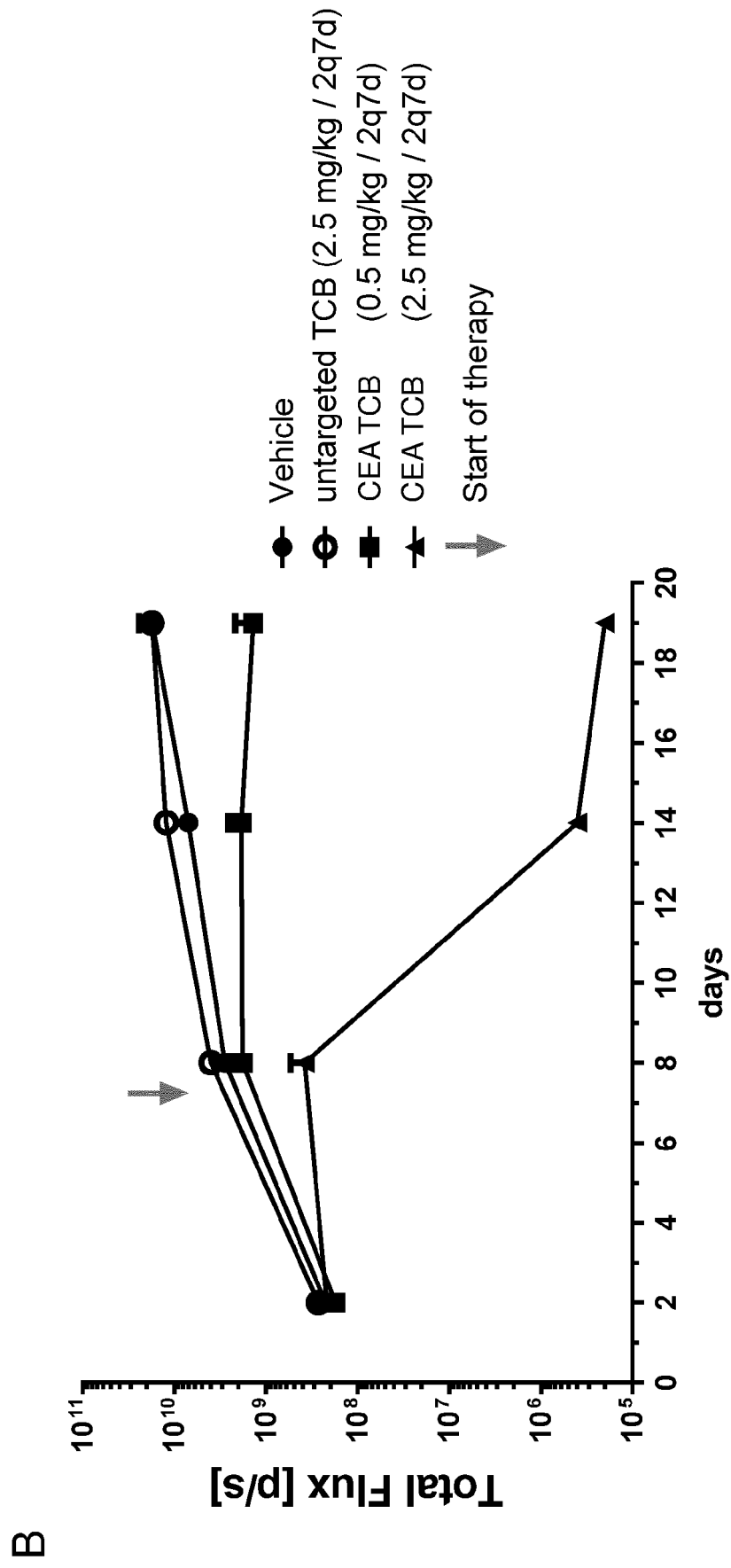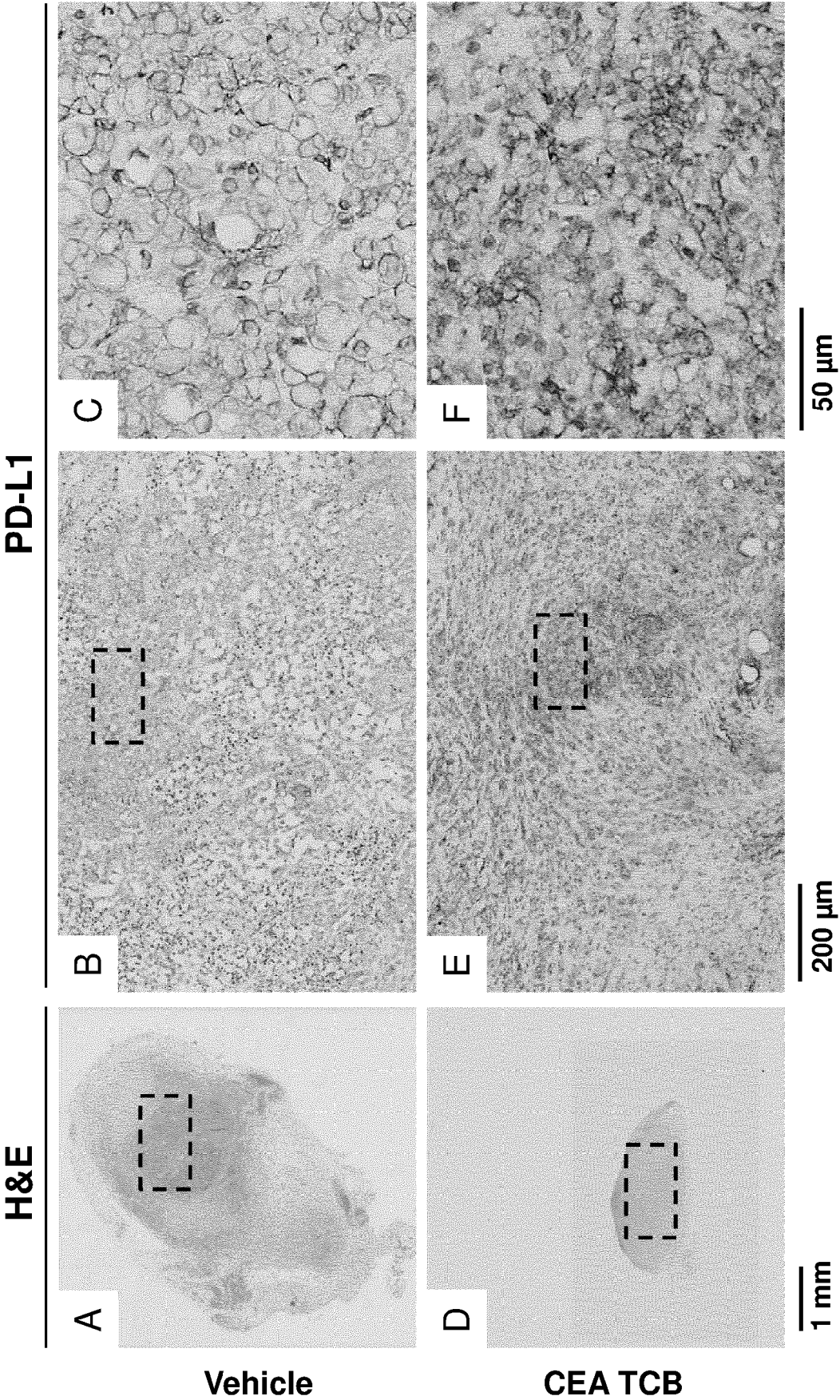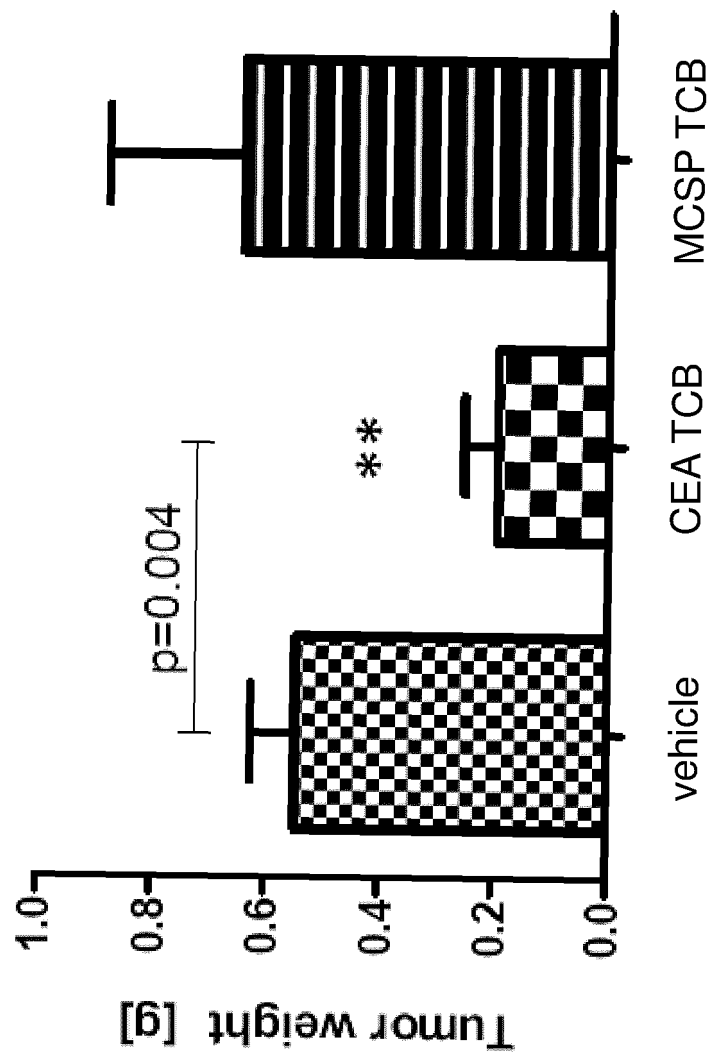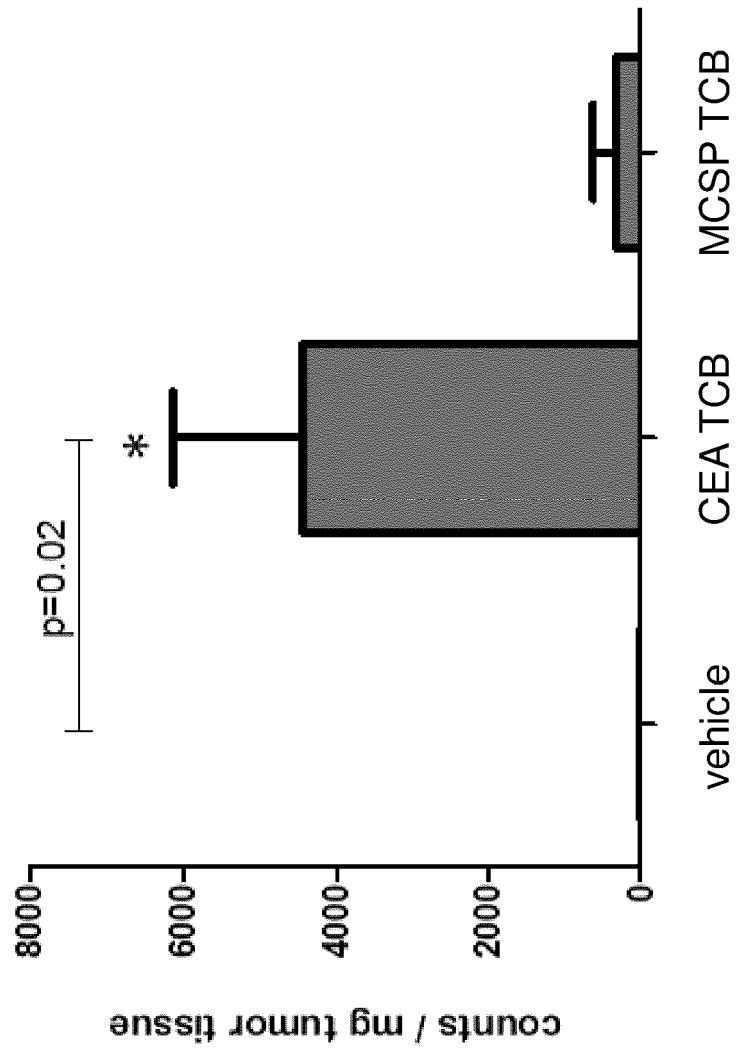
Figure 1

Figure 2

Figure 2

Figure 3

Figure 3

Figure 4

Figure 5

Figure 6
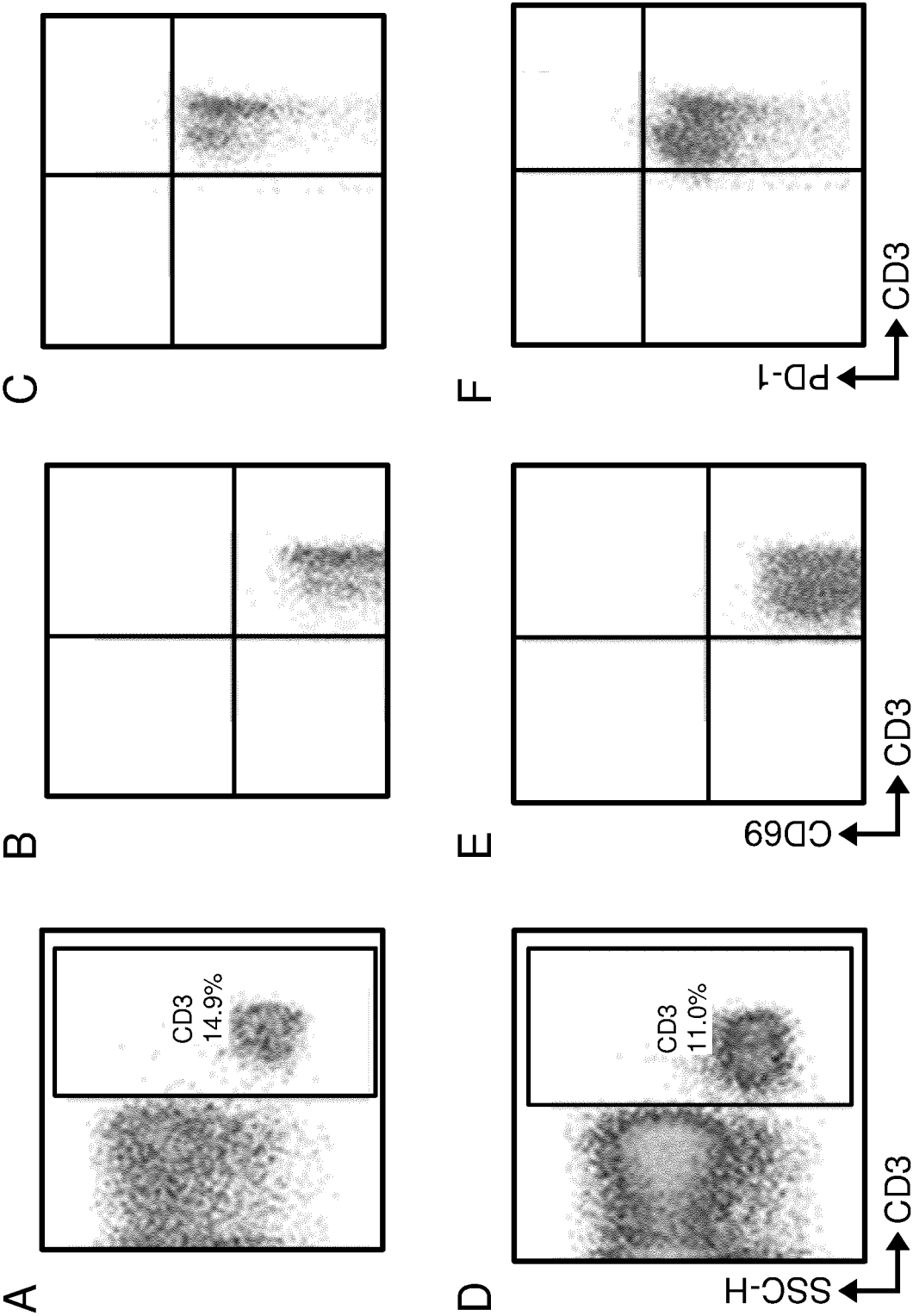
Figure 7

Figure 7

Figure 8
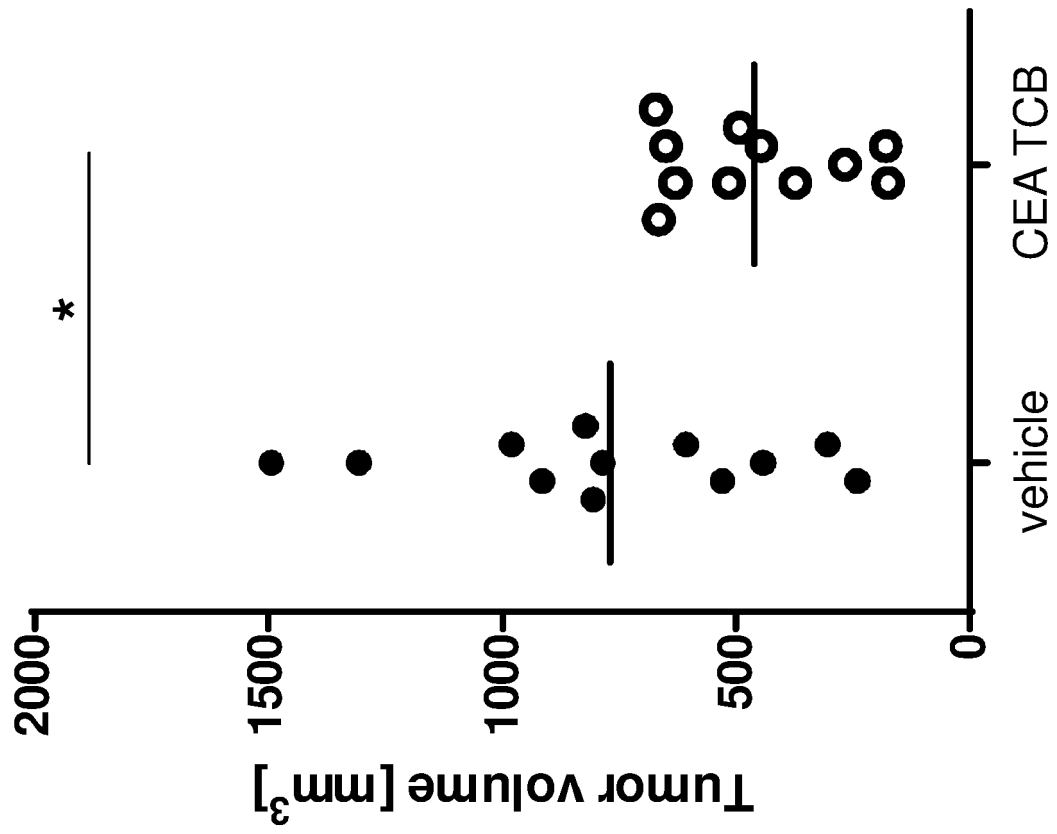
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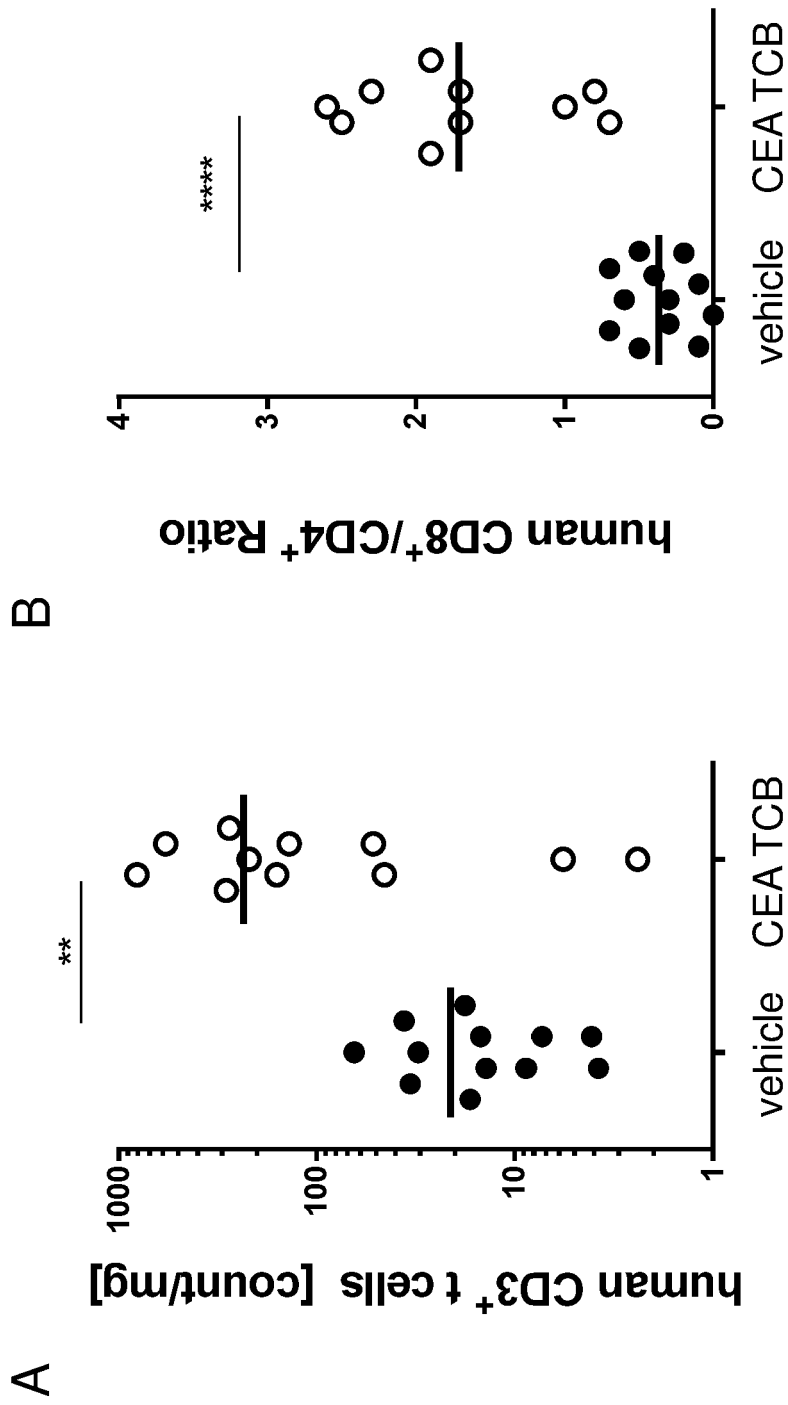
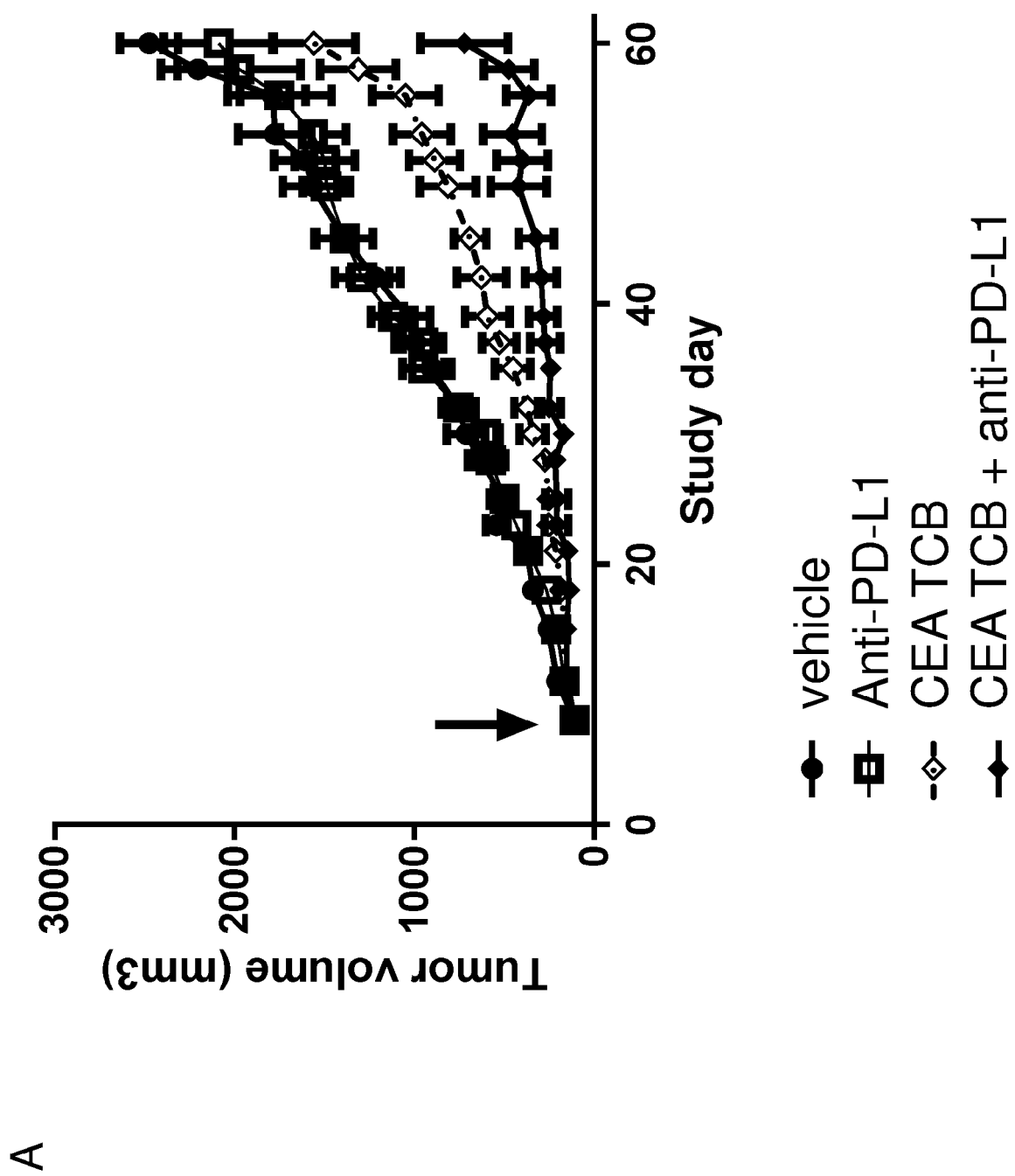Figure 9
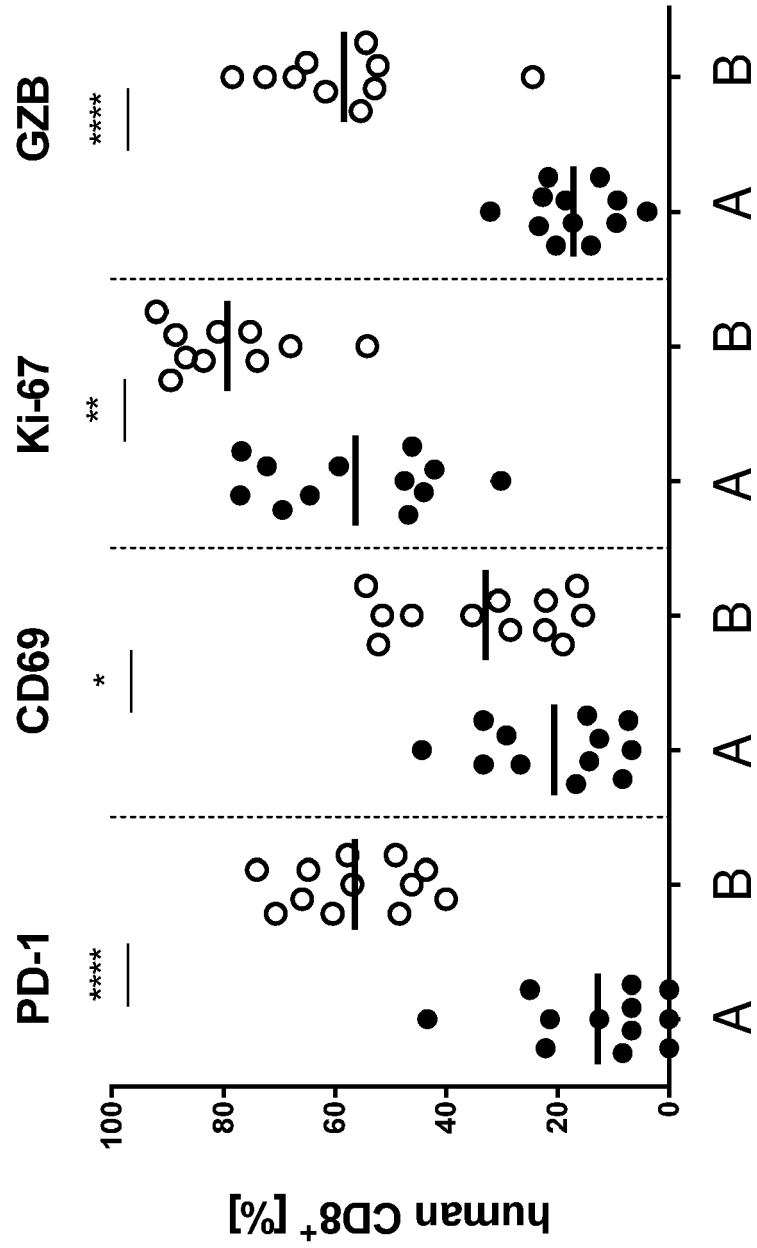
Figure 9

Figure 10

Figure 11
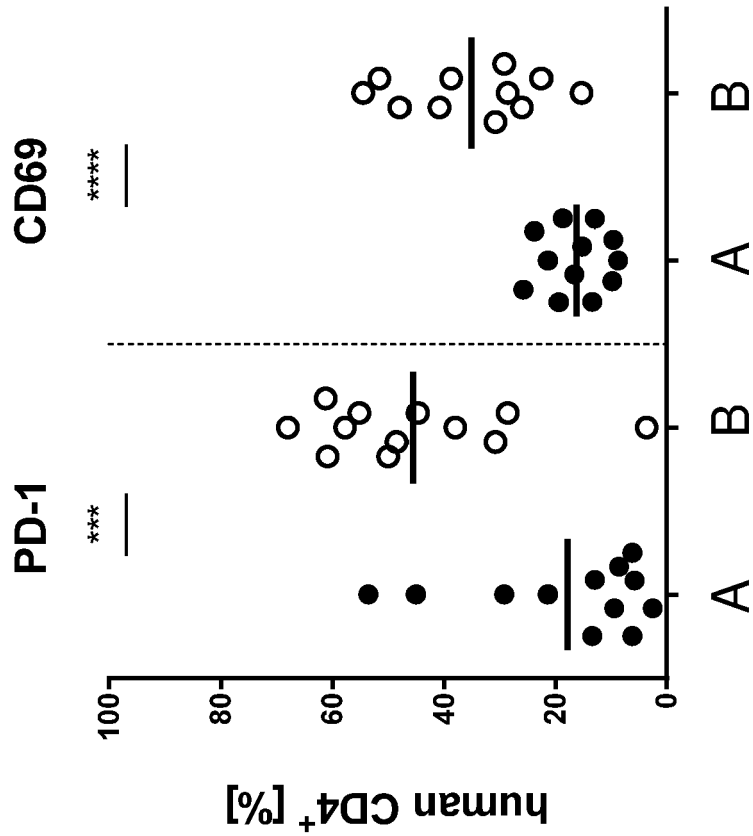
vehicle
Anti-PD-L1
CEA TCB
CEA TCB + anti-PD-L1
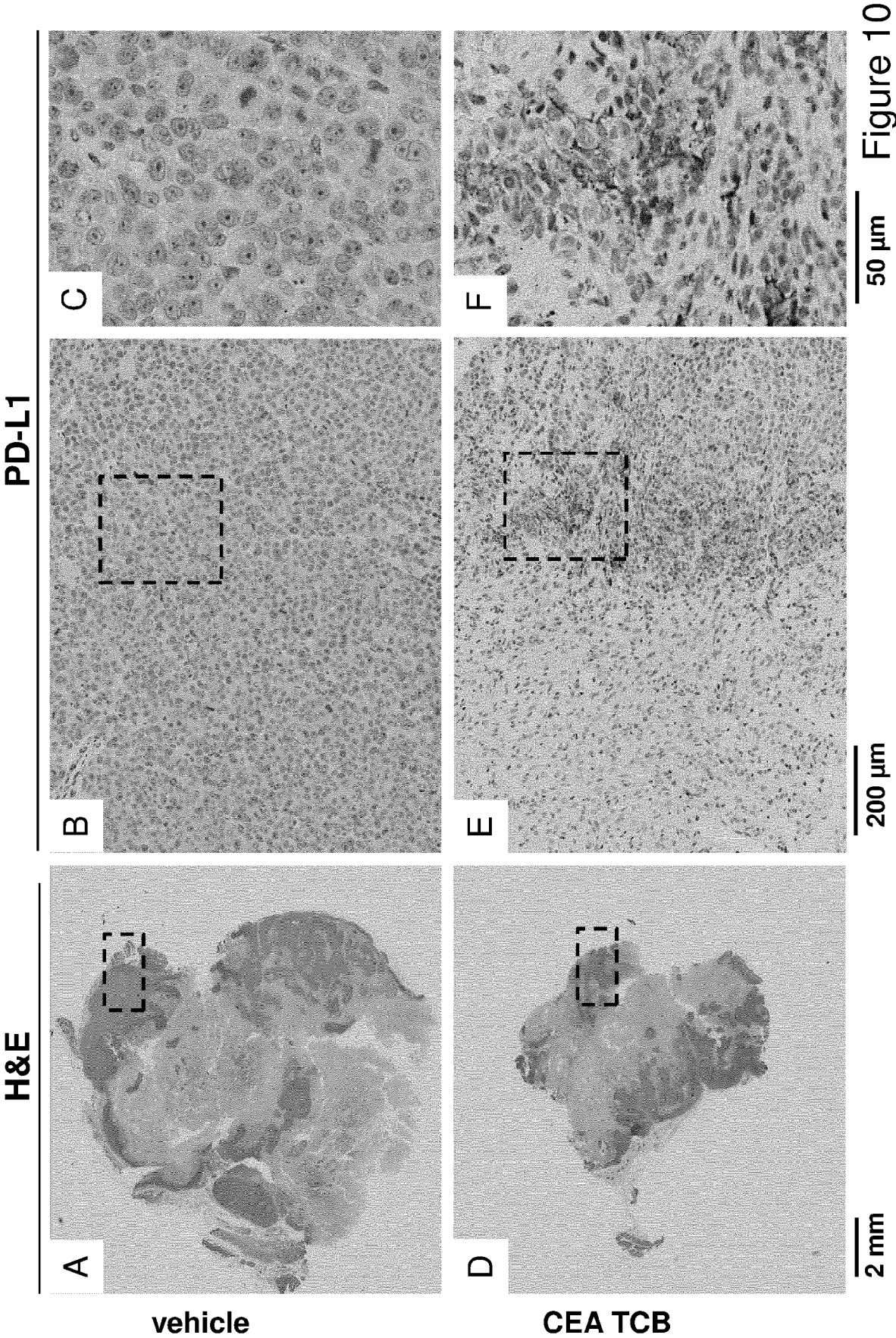
Figure 11

B
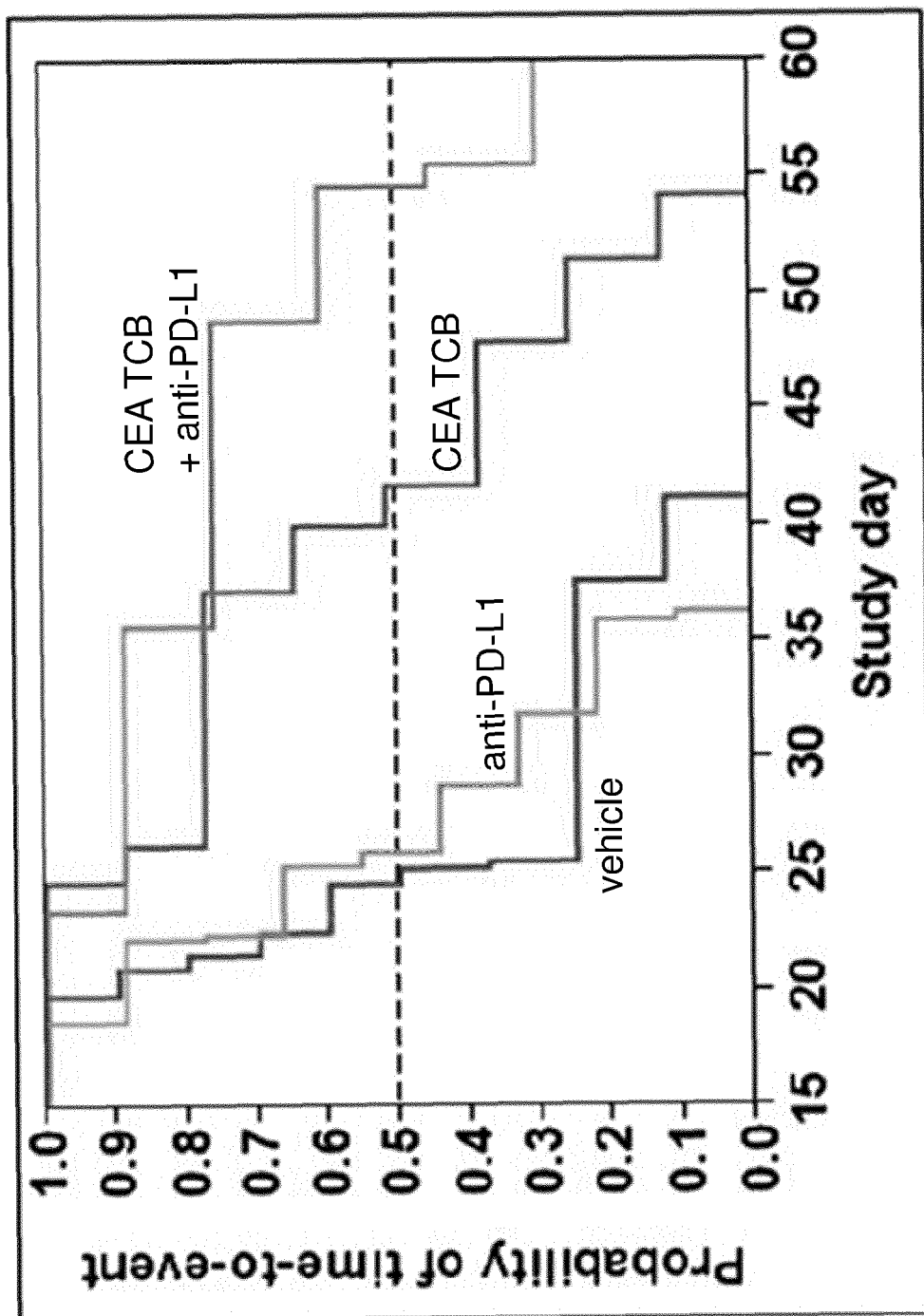
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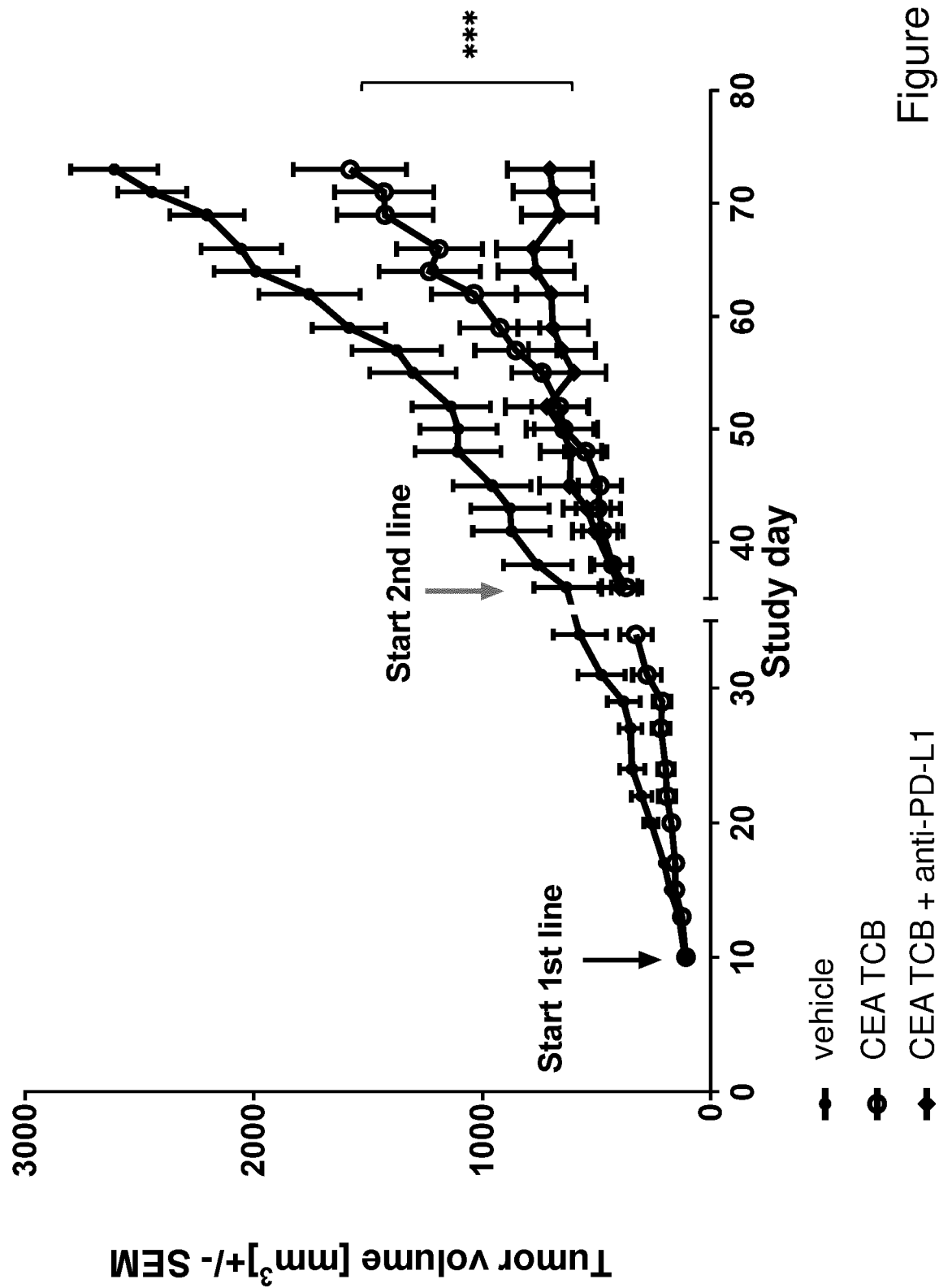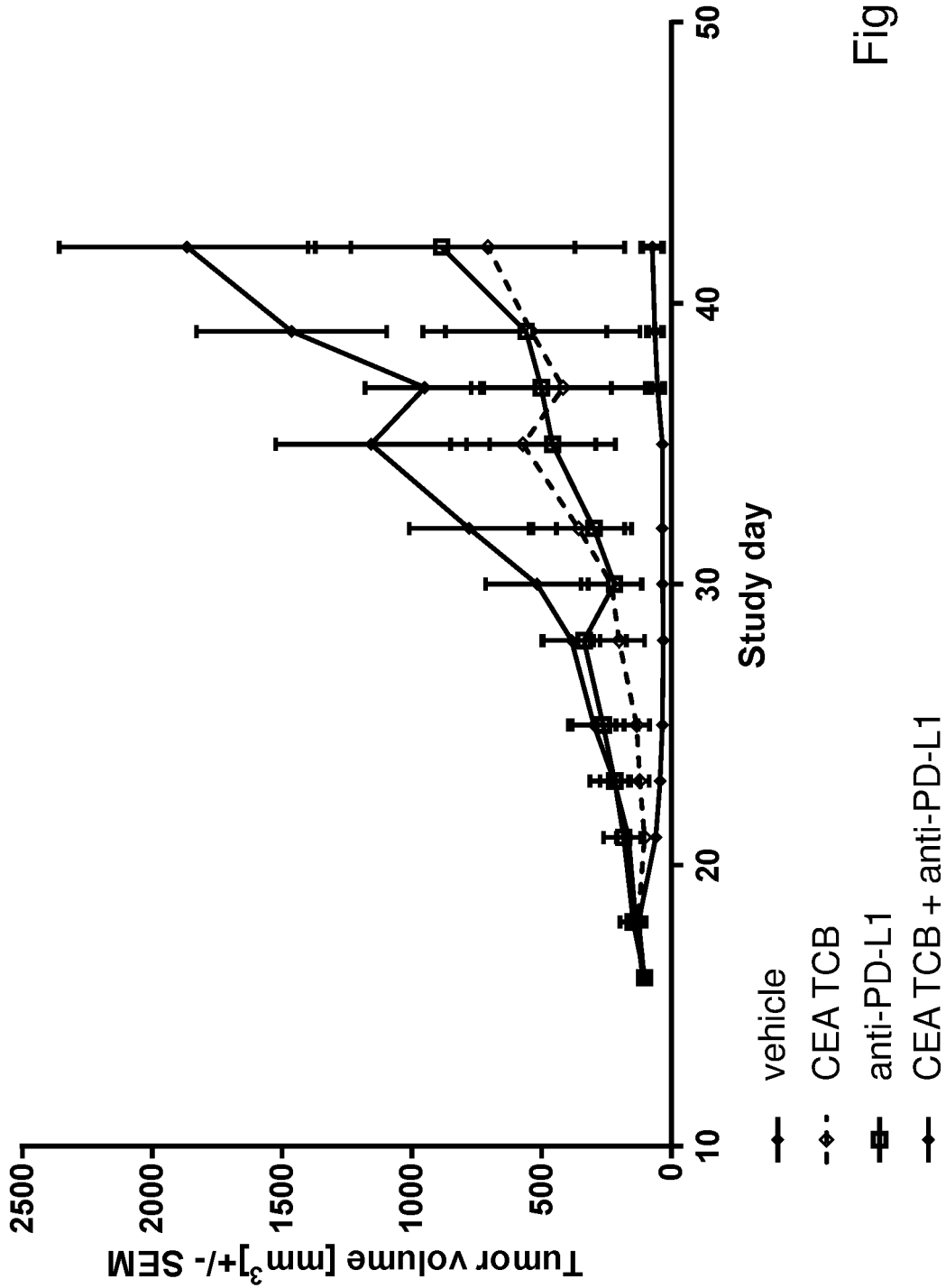
Figure 13

Figure 13

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	15 June 2021	Covone-van Hees, M

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Place of search	Date of completion of the search	Examiner
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